Patterns in flower visitation of flying insects in urban Christchurch

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By Lauretta Lynley Bensemann

School of Biological Sciences

University of Canterbury

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Bombus species visiting yellow *Helenium flexuosum* flowers in the Botanical Gardens, Christchurch.

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Abstract

In this project I studied the importance of pollinators in the reproduction of *Gastrodia* 'long column' and the preferences of New Zealand's native and exotic insect pollinators. This was done in order to determine the specialisation of insect pollination in urban Christchurch. This knowledge can contribute to crop pollination, conservation efforts, and an understanding of the main drivers of the common floral traits (small, white flowers) in New Zealand. The strength of the relationship between the common traits of the New Zealand flora and the preferences of the native invertebrate fauna is not known. Traditionally it had been thought that New Zealand's insects lack strong preferences, however recent work has not supported this. Changing landscapes worldwide have led to declines in pollinator numbers. Additionally, in New Zealand *Apis mellifera* numbers have declined as a result of the arrival of the varroa mite and it is important that alternative pollinating species for wild and agricultural pollination are identified. To address these needs I examined the abundance and preferences of insect pollinators in modified landscapes in and around Christchurch testing: the reliance upon pollinators by the undescribed native orchid *Gastrodia* 'long column', the preferences of New Zealand's native and introduced insect pollinators in an extensive observational study, the results of which I further tested using a subsequent manipulative experiment of petal colour (according to human vision) at the Christchurch Botanical Gardens

Sampling at the Christchurch Botanical Gardens, University of Canterbury, and Port Hills across a four month observation period (January – April 2012), revealed that native pollinators preferred white native flowers and exotic pollinators preferred not-white exotic flowers when data were grouped according to insect provenance. A more detailed visit-level analysis found that two native bees, *Hylaeus* and *Leioproctus,* showed a significant preference for native flowers; the exotic bees, *Apis mellifera* and *Bombus* species, preferred purple over white flowers; and *Melangyna novaezelandiae* (a native hoverfly) preferred white over yellow.

However, a series of experimental arrays to present controlled choices between pairs of flower types at the Christchurch Botanical Gardens (14 December 2012 – 22 January 2013) did not find significant preferences by native or exotic insect pollinators between white and yellow flowers. This may have been a result of the plant species chosen, as a correlation between pollinator preference and plant species has been shown elsewhere. Visitation to experimental arrays was both low and highly skewed, with over half of all visits made by *Lasioglossum* bees and 615 of the 669 visits made by native species. This may have meant that lack of significant results were representing the choices of native insects generally and *Lasioglossum* bees specifically.

A bagging experiment from 30 January 2012 – 16 February 2012 demonstrated the dependence of the undescribed native orchid species, *Gastrodia* 'long column', upon pollinating animals. Fruit set of most plants worldwide depends upon pollination (by wind or animal-transfer of pollen). In this case study final fruit sets were significantly reduced on bagged inflorescences, while open flowers had surprisingly high natural fruit sets (>75%). A week of observations (29 January 2012 – 6 February 2012) revealed that *Gastrodia* 'long column' was predominantly visited by *Lasioglossum* bees, and remarkably bee numbers were high enough in a residential property in the middle of Christchurch city for high fruit set on unmanipulated plants.

The results of my thesis indicate that pollinators are important in the reproductive system of *Gastrodia* 'long column', suggesting that other previously overlooked plant species may also rely upon insect pollinators. Furthermore, the relative importance of native pollinating insects is high for native plants even when examined in an urban setting. New Zealand pollinators have preferences for certain floral traits which show trends when grouped broadly, but vary when considered at the insect species level. This contrasts with traditional views of unspecialised insect pollinators which lack preferences in regards to the plants they visit. Further work which serves to increase current understanding of the underlying mechanisms of pollination specialisation in New Zealand may wish to focus on single insect species. By identifying particular preferences of pollinators and the underlying 'native' traits selected for, alternative options to crop pollination may be found, targeted management strategies implemented, and the strength of the relationship between pollinator preferences and the traits of the plants they visit determined.

Chapter 1

General introduction

New Zealand's native insect pollinators are thought to lack strong preferences that drive their visitation (Wardle 1978; Lloyd 1985; Robertson *et al.* 2005). This is unusual considering the empirical support of strong pollinator choice driving plant adaptations offshore (Pellmyr 2003; Bascompte & Jordano 2007). However, observational and experimental tests of pollinator preferences in New Zealand are few, a lack which has recently been addressed by Campbell *et al.* (2010; 2012). Their work in an alpine environment had mixed results, but did not rule out the possibility of pollinator preferences in New Zealand's native flower visiting insects. My thesis extends Campbell *et al.*'s (2010; 2012) work to include exotic insects in an urban environment in an attempt to increase the integrity of their findings across New Zealand. Specifically my thesis will test on a wide range of plant species whether pollinator preferences are linked to the common floral traits of New Zealand's plants and assess the importance of insect pollinators in the reproductive system of *Gastrodia* 'long column' in a case study. This first chapter serves to provide background information on pollination in New Zealand, with information particularly relevant to each data chapter provided in chapter introductions.

Pollination is an important ecosystem service worldwide and pollination in New Zealand often relies upon insect pollinators for its success. Some reproductive systems in New Zealand may also rely on insect pollinators that have not yet been identified, therefore understanding the interactions between plants and their pollinators is important when assessing the risk of pollination failure. New Zealand's pollinating insect fauna is made up of a combination of small native species, and larger exotics with increasingly complex social systems and behaviours (Newstrom & Robertson 2005). Flower constancy in insect pollinators can be useful in understanding pollinator behaviour because it helps to explain discrepancies between individuals of the same species. Therefore it is important to examine this process before pollination at the community level is investigated. The recent declines in *Apis mellifera* numbers contribute to the risk of pollination failure making this thesis timely. This is because it is more important now to understand insect pollinators and their preferences, so that management strategies can be appropriately targeted. Traditionally, native floral visitors are thought to not have strong preferences for particular floral traits. Therefore their native source plants are thought to not adhere to the parameters of current plant syndromes, as these syndromes have been defined based on evidence predominantly sourced from systems in the Northern Hemisphere (Newstrom & Robertson 2005). Recent evidence suggests that New Zealand's native insect pollinators do have some preferences in regards to the flowers they visit. Important ecosystem services such as pollination need to be understood so they can be managed and protected worldwide.

1.1 The mechanisms of pollination and the importance of mutualisms

Pollination is a valuable ecosystem service (any service provided by interacting organisms that benefits humanity), and one that is considered to be at risk (Klein *et al.* 2007). Pollination occurs when pollen is transferred to the stigma of a flower of the same species. This transfer is achieved

through a variety of means depending on the plant species (Wilcock & Neiland 2002; Osborne & Free 2003), but pollen is commonly moved via wind or insect (usually bee) vectors. Plants can also be pollinated by other animals such as birds, or vectors such as water; and possess adaptations which serve to facilitate pollination success (Osborne & Free 2003). The reproductive systems of plants can range from fully self-pollinated to complete self-incompatibility. Pollination in self-pollinating plant species can occur within the same flower (autogamy: pollen fertilises the stigma of the flower it was produced by) or between flowers on the same plant (Osborne & Free 2003). At the other end of the scale lies self-incompatibility where pollen must be transferred to the stigma of a second plant of the same species (cross-pollination). Mechanisms such as dioecy and spatial (herkogamy) or temporal (dichogamy) displacement exist in cross pollinating plants to reduce or prevent the occurrence of self-pollination (Osborne & Free 2003; Newstrom & Robertson 2005). Understanding the mechanisms of pollination is an important step in quantifying the importance of pollination services in New Zealand. This is particularly so when focusing on pollinator preferences, and allows management attempts to utilise natural behaviours.

Pollination services are important in New Zealand as New Zealand's plants are frequently dependent upon insect pollinators for successful reproduction (Newstrom & Robertson 2005). In one study of 358 indigenous plant genera, the pollination modes of plants were approximately 65.9% insect, 28.8% wind, 3.9% bird, 1.1% bat, and 0.6% water (Webb *et al.* 1999). This demonstrates the significance of insects for pollination services in New Zealand. This is the result of a number of factors such as unusually high levels of sexual dimorphism (Lloyd 1985) (~23% of seed plants are dimorphic (Webb *et al.* 1999; Newstrom & Robertson 2005)), functions which vary either spatially or temporally (herkogamy, monoecy, dichogamy), self-incompatible species, and plants that suffer inbreeding depression when self-pollinated (Newstrom & Robertson 2005). Note that these categories are not necessarily isolated, as a dichogamous species that is self-compatible may still self-pollinate (Newstrom & Robertson 2005). This was examined further by Newstrom and Robertson (2005) to determine true levels of pollinator dependence in New Zealand. Of the species analysed a little over 20% were found to be truly self-incompatible, with around 15% somewhat incompatible, and the remaining species being self-compatible. Despite the high number of self-compatible species in this limited dataset it appeared that selfing was still relatively rare. This gives an impression of the dependence on pollinating vectors as wind-pollinated species made up only a third of seed plants (Newstrom & Robertson 2005). The evidence that many floral species in New Zealand are highly dependent upon pollinators demonstrates New Zealand's vulnerability to a decline in pollinator numbers (Newstrom & Robertson 2005), and many more plant species are yet to be examined.

Pollinator services may also be important in systems that are not thought to rely on them. By examining possible mutualisms in less conspicuous species it can be determined whether pollinators also choose to visit unexpected (and less attractive) species. There are five *Gastrodia* taxa in New Zealand (Scanlen & St George 2011). One of these is *Gastrodia* 'long column', of which little is known about its pollination system. A study which looked at the closely related *Gastrodia cunninghamii* in the North Island of New Zealand over a 2 month long flowering period had conflicting results around *Gastrodia cunninghamii's* breeding system. This species has been thought to be autonomously selfing. However, in their study seed production failed after plants were self-pollinated by hand, but seeds were produced when plants were able to self-pollinate themselves

and when cross-pollination was performed by hand (Lehnebach *et al.* 2005). Their orchid species was found to have no discernible scent to human senses although stains taken indicated the potential for scent production (Lehnebach *et al.* 2005). Aphids were observed within *Gastrodia cunninghamii*, allowing for the possibility of some role in pollination although their study did not pursue this hypothesis any further (Lehnebach *et al.* 2005). Morphologically *Gastrodia cunninghamii* appears adapted for autonomous autogamy, but may be producing scent (which would be to attract pollinators), and seed production failed after hand self-pollination. Therefore conclusions cannot yet be drawn. Similarly, *Gastrodia* 'long column' is relatively inconspicuous, with a stem from which all flowers extend (Rolfe 2010). This stem is brown and flowers have a long column and a yellow labellum apex, distinguishing them from other closely related species (Rolfe 2010). By determining whether *Gastrodia* 'long column' relies upon pollinators, and which pollinators (if any) will visit, a greater understanding of the importance of pollinators in New Zealand's orchid breeding systems can be established.

1.2 New Zealand's insect pollinators

New Zealand has a mix of native and exotic insect pollinators (see Donovan 2007 for a review of New Zealand's bees: Hymenoptera). Of the native species, native bees are much more inconspicuous than bees exotic to New Zealand. *Leioproctus* species (Colletidae) are the largest of these native bees, and the most "bee-like" of New Zealand's insect fauna (Donovan 2007). *Hylaeus* species (Colletidae) tend to be much smaller and less conspicuous, and are thought to visit a range of native and exotic plant species, to the point where nest sites, rather than food resources, are likely a limiting factor in population sizes (Donovan 2007). *Lasioglossum* bees (Halictidae) are often the smallest of the native bees, with much variation in the plant species they visit. Flies, particularly syrphid flies (Campbell *et al.* 2010), are also important in insect pollination in New Zealand, with many plants displaying suites of traits that are preferred by them (Newstrom & Robertson 2005). Exotic insect pollinators also contribute to pollination in New Zealand (Kelly *et al.* 2006). Eight exotic bee species have been deliberately introduced to New Zealand since the 1830's (Howlett & Donovan 2010). Of these, *Apis mellifera*, the honey bee, is found worldwide and is considered to be one of the most useful pollinators for agriculture (Howlett & Donovan 2010). Caution should be used when such claims of importance are made however, as these claims have been said to lack empirical evidence (Ollerton *et al.* 2012). *Apis mellifera* is classed as a generalist forager and is believed to visit nearly all native and exotic plants found in New Zealand (Donovan 2007). Of the four bumblebees found in New Zealand; *Bombus terrestris* is also considered to be fairly general in its New Zealand visitation while *Bombus hortorum, Bombus ruderatus,* and *Bombus subterraneus* are more limited in scope of visitation. *B. subterraneus* is rare in New Zealand and possibly extinct in its homeland, making it perhaps also a focus for conservation efforts (Donovan 2007). It is worth noting that *Bombus* species are notorious for nectar robbing (Donovan 2007), and so may not always provide beneficial services to plants. There are a number of other species which are not covered here. The effectiveness of these pollinators varies.

To be an effective pollinator an animal should do a number of things. Firstly, a pollinator must visit a number of different plant individuals of the same species. Secondly, a pollinator must be mobile, the more so the better. Lastly, a pollinator must be able to carry pollen between plants by brushing against anthers and collecting pollen and then making contact with the stigmas of receptive flowers

to transfer the pollen (Osborne & Free 2003). Bees are considered particularly effective insect pollinators because of their mobility and hairy bodies (Osborne & Free 2003). Some visitors are less useful as vectors, while the preferences of others may reduce their pollinator 'usefulness' as they do not transfer pollen to conspecifics (Lau & Galloway 2004). Plant species may group phenotypic traits which results in more consistent visitors, which in turn increases the effectiveness of their pollinators. Effectiveness is an important qualitative term because it shows why plant traits may suit some pollinators much more so than others.

1.3 Plant syndromes and their relevance to the New Zealand flora

Floral syndromes have been suggested as a way to predict the groups of pollinators that will visit plant species. This concept proposes that plant species are visited predominantly by certain groups of animal species because they have suites of traits which are preferred by these visiting groups (Chittka & Thomson 2001; Robertson *et al.* 2005). Insect pollinated floral syndromes include large flowers that often have a combination of cues designed to target an insect's innate preferences. Plants also provide rewards which encourage pollinators to return to visit plants of the same species through reward association (Osborne & Free 2003). The importance of floral syndromes has been reported as overstated in the southern hemisphere (Hingston & Mc Quillan 2000; Newstrom & Robertson 2005). In a Tasmanian study which tested the predictability of visitors based on the apparent pollinator syndrome of the plant, very few species were exclusively pollinated by the expected animal (birds in this case), while the rest were all visited by more than one group of pollinators (Hingston & Mc Quillan 2000). This lack of exclusivity was emphasised by the inability of the authors to apply the concept to a number of plant species with generalised traits, and implied that none of the pollination syndromes applied to the plants of this study could be fairly used as an accurate predictor of its visitors (Hingston & Mc Quillan 2000).

In New Zealand, pollination syndromes may be less relevant due to New Zealand's apparent high levels of generalisation both in the pollinating fauna, and the pollinated flora (Newstrom & Robertson 2005). This was demonstrated to some degree in a study which examined two New Zealand mistletoes; *Peraxilla tetrapetala* and *Peraxilla colensoi*. Both of these species fit neatly into an ornithophilous pollination syndrome, often red or orange flowers which appear to exploit bird preferences, behaviours, and morphologies (Robertson *et al.* 2005). This study observed short-tongued bees successfully pollinating one of these species, *P. tetrapetala* (Robertson *et al.* 2005). The results of this study suggested that bees were able to replace birds as pollinators to some extent, despite the ornithophilous syndrome of the mistletoe flowers. The contrast in pollination success of two similarly ornithophilous species which were visited by bees show generalisations around floral syndromes should be made with caution. There is much debate on both the syndrome prescriptiveness and tightness of plant-pollinator associations (Chittka & Thomson 2001). Because fewer plant-pollinator relationships have been documented here than elsewhere in the world much is still to be learnt about floral syndromes in New Zealand (Newstrom & Robertson 2005). Therefore it seems reasonable for the duration of this study to make no predictions about flower visitors based upon the apparent syndrome of the flower species chosen.

1.4 Flower constancy

Flower constancy occurs when foragers repeatedly visit flowers of the same or a few plant species even when other suitable flowers are readily available (Gegear & Laverty 2001). This foraging strategy is important when considering the relationship between plant species and their pollinators because it influences the transfer of pollen between conspecific plants (Chittka *et al.* 1999). Animals which exhibit flower constancy may preferentially visit certain species, or morphs within a species, even when other perfectly suitable alternatives are more available (Waser 1986; Chittka *et al.* 1999; Menzel 2001; Osborne & Free 2003). This constancy may be the result of a limited ability to remember more than a few flower morphs at a time, or that learning more morphs is too costly and time-consuming (Waser 1986; Menzel 2001). A characteristic of flower constancy that is worth noting is that this occurs at the individual pollinator level. This means that pollinating animals within the same species may specialise on different flower morphs to one another because each individual animal will have encountered different flowers over their lifetime and stored these experiences (Waser 1986; Chittka *et al.* 1999). Therefore two bees from the same hive may consistently visit different flowers in a foraging patch and so flower constancy is important when investigating flower visitation by pollinators.

Two key hypotheses converge to explain the flower constancy of pollinators. These are Darwin's interference hypothesis and the search image hypothesis. Darwin's interference hypothesis states that it is advantageous from a plant's point of view for foragers to specialise on a single pollinating species (cross-pollination), while it benefits the animal to specialise on plants because experience leads to reduced handling times (Gegear & Laverty 2001). The search image hypothesis asserts that foragers 'sieve' through high volumes of visual information rapidly by specialising on 'runs' of one prey type (Gegear & Laverty 2001). Many authors do not believe that these two concepts are mutually exclusive, and a trade-off between accuracy and speed is thought common (Chittka *et al.* 2003; Chittka *et al.* 2009). Another hypothesis for flower constancy is Gegear and Laverty's (2001) 'trait variability hypothesis'. This idea is again interlinked but is broader in its focus. Here they suggest that flower constancy may be the result of a broad suite of factors rather than simply time and single floral traits. More explicitly, pollinators make decisions based on traits such as floral colour, as well as a second trait like flower size which may or may not be correlated with the first. This is supported with evidence that pollinators are more likely to be choosy if their target species differ in multiple traits, and act inconsistently when only one trait varies (Gegear & Laverty 2001). Flower constancy is important because it may serve to explain within-species variation in pollinator preferences, and the risk of reproductive failure to plants.

1.5 The risk of pollination failure and the loss of pollinators

Pollination success depends upon a number of factors. The pollen quality and quantity of a flower is important, as is its timing and colouration. These attributes can influence pollinator attraction and the successful fertilisation of a receptive stigma (Wilcock & Neiland 2002). Secondly, pollination can depend on the pollinator as if there are too few pollinating individuals or visitation is erratic then pollen transfer is reduced. Thirdly, pollination success is influenced by the whole plant as its attractiveness and rewards can entice pollinators and encourage their return to conspecifics, while their accessibility and complexity may prevent some pollinators from visiting. Finally the wider

community can have an influence on pollination success because diversity of species or fragmentation of the flora can affect crossover (Wilcock & Neiland 2002). Pollination success varies naturally. However, human activity has meant that the risk of failure may no longer be fluctuating in natural patterns and more consistent failure may be likely (Wilcock & Neiland 2002). Failure in New Zealand is difficult to assess because of the reliance on a broad suite of animal pollinators. However, evidence suggests that New Zealand avifaunal pollination is at a greater risk of failure than bird dispersal (Kelly *et al.* 2010). Their paper recently reviewed bird pollination and dispersal in New Zealand, finding that of the ten ornithophilous plants tested eight showed that seed production was significantly limited. Pollination limitation indices were higher than the global average (Kelly *et al.* 2010). A similar review of plant-insect mutualisms is timely. Pollination limitation in New Zealand has been examined. In a study of 33 populations (which included a range of breeding systems from across New Zealand), 21 were found to be suffering from significant pollen limitation, with some seed production as low as 10% of that of hand pollinated individuals (Newstrom & Robertson 2005). Another study looked at a group of New Zealand shrubs and found pollen limitation across hermaphroditic and self-incompatible taxa. Of these, those that were pollinated by insects were consistently placed as more vulnerable than those that were wind pollinated; demonstrating the sensitivity of more specialised, self-incompatible species that rely on animal vectors (Merrett *et al.* 2007).

Evidence around the world is accumulating that pollinator numbers are suffering reductions (Osborne & Free 2003). Changing environments through land clearing, climate change, invasive species and agricultural intensification (Wilcock & Neiland 2002; Osborne & Free 2003; Diekötter *et al.* 2006; Klein 2011), along with the use of harmful chemicals for increased production; have led to the loss of diverse habitats. This may have resulted in the loss or reduction of many pollinating insects (Osborne & Free 2003). While the reduction of crop-pollinating Hymenoptera numbers such as *Apis mellifera* and *Bombus* species, along with some butterflies, have been clearly demonstrated, the impact of human activities upon other less conspicuous species is not certain (Wilcock & Neiland 2002; Ghazoul 2005). In some cases this impact may be positive (Ghazoul 2005). Because of the difficulty in assessing flow-on effects and ambiguity of research, more empirical data on the behaviours and relative abundances of less conspicuous insect species is needed before a pollination crisis claim should be made (Wilcock & Neiland 2002).

1.6 Reductions in *Apis mellifera* numbers in New Zealand

Traditionally agriculture has relied heavily upon the honey bee, *Apis mellifera,* for crop pollination both in New Zealand and overseas (Howlett & Donovan 2010). Recently however, *Apis mellifera* numbers have begun to decline with the arrival of the varroa mite (*Varroa destructor*) to New Zealand in 2000. Because of this it is necessary to assess other possible insect alternatives to crop pollination, and to consider the wider impacts to wild pollination (Howlett & Donovan 2010). Estimates of the cost of such a loss over a 24 year period, along with attempts to control the mite, range in the hundreds of millions (Howlett & Donovan 2010). Such an impact on New Zealand's economy would be harmful. However, the honeybee is not the only exotic bee to have been introduced to New Zealand to aid with agricultural pollination (Howlett & Donovan 2010), and it is possible that its importance may have been overstated in some systems (Ollerton *et al.* 2012).

Other insect pollinators should be considered carefully when determining the impact of the arrival of the varroa mite. This is particularly important because a number of agricultural industries in New Zealand are solely dependent upon the honeybee for pollination services. Thus a loss without identified pollinator alternatives could be damaging to the New Zealand economy (Howlett & Donovan 2010). Generalist exotic species such as *Bombus terrestris* and *Megachile rotundata* may be able to provide these alternatives, and work on man-made nesting boxes for *Bombus terrestris* has been successful in its early stages although more experimental work is needed (Howlett & Donovan 2010). There are difficulties in using bumblebees, however. These bees have much smaller colonies than *Apis mellifera* and so more nests may be needed. However, an advantage is they are able to forage at lower temperatures than many other species (Howlett & Donovan 2010). The use of the Lucerne leafcutting bee (*Megachile rotundata*) is more limited because it has been difficult to establish in great numbers and it is much more sensitive to cool or windy climates (Howlett & Donovan 2010). Other introduced bees such as *Nomia melanderi* and *Osmia coerulescens* have also been considered. Native bees and flies have not received the same levels of attention. An issue for alternative species is that they are not as general in their foraging habitats as *Apis mellifera* and so informed decisions when selecting the appropriate pollinator will need to be made (Greer 1999). While *Apis mellifera* numbers are declining a pollination crisis should not be claimed until all alternative means of pollination have been examined in depth. Native pollinators may show preferences for suites of traits exhibited by some crop plants, and so understanding their preferences may mean they can be used to supplement pollination previously relied upon by domestic exotic-bee colonies.

1.7 The relationship between plant traits and pollinator preferences

Plant traits are often tightly linked to the preferences of their pollinators (Bascompte & Jordano 2007) and strong preferences may drive speciation in some flowering plants (Gegear & Burns 2007). Plants attract pollinators through attractive displays (Osborne & Free 2003), while pollinators make their selections based on a number of (often linked) cues such as colour (Osborne & Free 2003) and resource quality (Robertson *et al.* 1999). Therefore conspicuous traits that are linked with quality resources will be selected for by mobile visitors. New Zealand's common flower traits include small, white flowers, often dish-shaped, with simple easily accessed morphologies (Lloyd 1985; Webb & Kelly 1993; Newstrom & Robertson 2005). Insect pollination in New Zealand has traditionally been considered reasonably generalised with unspecialised pollinator species (Lloyd 1985; Newstrom & Robertson 2005). However, recent evidence predicts that New Zealand's native insect pollinators will have some preferences in regards to the flowers they visit, but this is not the predominant driver of floral evolution in New Zealand. Campbell *et al.* (2010, 2012) found that they could not reasonably conclude that New Zealand's pollinators lack colour preferences. Some native invertebrate species were found to occasionally prefer colours, a finding influenced by both plant species and spatial scale. Therefore investigating the preferences of New Zealand's insect pollinators with this new evidence is important so that fresh assessments of pollinator choice in New Zealand can be made.

1.8 Thesis objectives

The overall objective of this thesis is to determine the importance of pollinators in less studied systems, and whether these pollinators have preferences for particular plant traits. From this I

provide some evidence of a relationship between the common floral traits of New Zealand native plants and the preferences of their native pollinators. Management strategies which hope to increase native faunal numbers can select appropriate plant substrates using this information, and may also select possible pollinator replacements for *Apis mellifera*. Because I also include exotic insect pollinators in this study, predictions around changes to the direction of selection on angiosperm traits in New Zealand as a result of their preferences are also made.

To achieve this, observational work across four sites in Christchurch is compared to experimental arrays set up at one of the sites. Three of the four sites are in Christchurch City. These are the Botanical Gardens, the University of Canterbury, and a residential property near the city centre. The last site is the Sign of the Bellbird in the Port Hills which is city-adjacent and contains secondary native forest. A combination of experimental and observational work is used because this allows results to be compared in natural and controlled systems so that certain variables are controlled for. I test the importance of flower colour, plant provenance, climate, plant species, and site, to pollinator visitation through instantaneous counts, as well as pollinator importance through a bagging experiment. All colour phenotypes in this thesis are graded according to human vision. Therefore all references to colour data as collected and assessed in this thesis mean according to human vision only.

The following specific questions are investigated:

1. Do New Zealand's pollinators have clear preferences in regards to their target plant's provenance, location or display?
2. Do New Zealand's pollinators make foraging decisions based on the colour of the flower?
3. Does *Gastrodia* 'long column' depend upon pollinators for successful fruit-set?

Chapters 2-4 are written as stand-alone chapters and all chapters are analysed along with their contributions to the understanding of New Zealand insect pollination in the concluding chapter.

Chapter 2

The preferences of New Zealand's flower visiting insects

2.1 Introduction

This chapter will test the predictors of pollinator visitation in urban New Zealand. Plants and their animal pollinators are often in a mutualistic relationship which drives the adaptation of some floral traits (Pellmyr 2003; Bascompte & Jordano 2007). Therefore localised floral adaptations are often linked to the preferences of the pollinators who visit them. New Zealand's floral displays follow some noticeable phenotypic trends; traits commonly seen are small, inconspicuous flowers (Webb & Kelly 1993), often white (~60%), with dish or short tube shapes (Lloyd 1985; Newstrom & Robertson 2005). Previous authors have determined that the New Zealand invertebrate pollinating fauna consists of relatively small and unspecialised insects (Donovan 2007) which are hypothesized to lack strong preferences in regards to floral visitation (Wardle 1978; Lloyd 1985; Robertson *et al.* 2005). If New Zealand's pollinators lack strong preferences then the adaptations of New Zealand's native flowers may to conform to other selection pressures such as heat tolerance or water conservation, rather than waste energy on large colourful displays that elsewhere serve to attract pollinating visitors.

Considering the relationship between plants and exotic pollinators is also important when examining the preferences of New Zealand's pollinators. A number of exotic pollinators are now established in New Zealand and these pollinators may have important implications for the future of New Zealand's flora. This is because their preferences may change the direction of selection on New Zealand's plants (Brockerhoff *et al.* 2010) towards phenotypes preferred by exotic pollinating species. The idea of localised pollinator preferences has led to the two hypotheses: New Zealand plants are resistant to invasion (Brockerhoff *et al.* 2010), and conversely, that exotic insect preferences encourage the proliferation of weed species (Goulson 2003; Hanley & Goulson 2003; Howlett & Donovan 2010).

In order to establish the relationship between floral phenotypes and pollinator preferences in New Zealand it would be useful to assess the relative abundances of native and exotic pollinators at sites with different plant communities, determine whether pollinators preferentially visit plants of the same provenance, and whether the preferences of New Zealand's native insect pollinators are linked with the common phenotypes of New Zealand's native flora.

The interactions between plants and their pollinators

Plant-pollinator signals

Pollinators rely on signals from flowering plants to direct their visitation. Plants attract pollinators with bright displays, attractive scents, shapes, sizes, and enticing rewards (Osborne & Free 2003). These rewards serve as a way to encourage the return of a pollinator, either to the same plant or other individuals of the same species, thus resulting in successful pollination. Because bees are dependent upon flowers for their nutrition (pollen and nectar are their only sources of carbohydrates and protein (Osborne & Free 2003; Newstrom & Robertson 2005)) they are frequent

visitors, which has led to bees being identified as the most important pollinating group worldwide (Newstrom & Robertson 2005).

Invertebrate pollinators are mobile and able to make decisions based on the information they are receiving. The ability of flower visitors to assess the quality of rewards received has been investigated. In low density greenhouse trials at the University of Exeter, UK, *Bombus pratorum* has been shown to discern between pollen rewards (cytoplasmless and viable) when a plant still has most of its pollen available by foraging upon high quality plants (Robertson *et al.* 1999). In outdoor trials *Bombus* taxa were shown to be able to discriminate between high and low quality patches. This cognitive ability to assess reward quality means that plants which send clear signals and provide ample rewards will be visited relatively more often than less conspicuous, low-rewarding plant species. A study in Wiltshire, southern England, assessed the correlation between plant breeding systems and pollen quality. In species which obligatorily used insect visitors for pollination, pollen quality was significantly higher (Hanley *et al.* 2008). This shows the link between plant rewards and pollinators, demonstrating the importance of pollinator choice in driving adaptations in the plants which rely on them.

Plant provenance and the provenance of their visitors

A number of Australian studies have tested the relationship between the provenance of flowering plants and the provenance of bee visitors with conflicting results. In Tasmania it was found that *Bombus terrestris* was far more abundant in areas dominated by introduced plant species, a result attributed to their preference for exotic flowers (Goulson *et al.* 2002). Introduced bees have a strong preference for introduced flowers in both New Zealand and Tasmania, and in particular, European bees are the primary pollinators of European weeds (Goulson 2003). Conversely, Goulson *et al.*'s (2002) study and Goulson's (2003) review were followed by a later paper which found little evidence to support the claim that *Bombus terrestris* preferentially visited exotic flowers in Tasmania (Hingston 2005). They suggested earlier results were strongly biased because numbers hadn't been corrected in light of the difference in abundance of native and exotic pollinators (Hingston 2005). A recent review examined the existing evidence and supplemented it with another season of data, concluding that *Bombus terrestris* has 'extensively invaded the Tasmanian native vegetation' and is nesting in native parks in every Tasmanian region (see Hingston 2006 for a review of the conflicting evidence)(Hingston 2006). This may suggest that in New Zealand exotic bees will willingly forage on both native and exotic flowers, however, as the New Zealand flora is unique no generalisations based on behaviour elsewhere should be made.

Pollination in New Zealand

The New Zealand flora

New Zealand's separation from Gondwanaland over 80 million years ago has led to the adaptations of its native species and high levels of endemism (Cooper & Millener 1993; Webb & Kelly 1993; Newstrom & Robertson 2005). While the flora has a high percentage of endemic species, genera are frequently shared with Australia (Cooper & Millener 1993). More recent arrivals are also often of Australian origin because of the direction of the ocean currents and winds (Cooper & Millener 1993). The arrival of humans in New Zealand ~1000 years ago, and again ~200 years ago, is known to have

had drastic impacts on the native flora. Prior to this time New Zealand was thought to be dominated by forest (Holdaway 1989).

The most common features of the New Zealand flora (outlined in the first paragraph of this chapter) contrast with large, intricately shaped, and brightly coloured displays found elsewhere in the world (Osborne & Free 2003), and are attributed to a generalist pollinating fauna (Lloyd 1985). Over 80 % of New Zealand's native flora is endemic and half of the current flora is made up of naturalised exotics (Newstrom & Robertson 2005; Howlett & Donovan 2010). Plants can be pollinated via a number of vectors such as insect or wind (Newstrom & Robertson 2005) and floral traits typically fit an entomophilous (insect-pollinated) flower syndrome. However, it is not thought that plant syndromes (as they are currently defined) are predictive of pollinator visitation to the New Zealand flora (Newstrom & Robertson 2005). Authors have proposed that New Zealand's pollinating fauna (particularly the fly fauna (Newstrom & Robertson 2005)) has played a major role in the direction of flower evolution (Lloyd 1985), although colours such as white might be indirectly selected for as a result of pleiotropic effects on other traits (Campbell *et al.* 2012). Worldwide, other physiological factors such as drought tolerance have been shown to direct floral evolution (McKee & Richards 1998; Schemske & Bierzychudek 2001; Armbruster 2002; Coberly & Rausher 2003; Gould 2004).

The intention of this chapter is to determine whether New Zealand's native pollinators show preferences for the traits typical of the New Zealand flora, and exotic pollinators for traits more typical of exotic plant species. If native pollinators do show strong preferences for small white flowers then perhaps they have played a role in selecting for these adaptations.

New Zealand's pollinating fauna

The New Zealand insect pollinating fauna has a range of native species, with most important contributors falling into one of Hymenoptera, Diptera, and Lepidoptera. A number of exotic Hymenoptera were deliberately introduced to New Zealand for crop pollination, and a few species across other orders also play a role in pollination. Insect pollination in New Zealand is considered reasonably generalist with unspecialised pollinator species (Lloyd 1985; Newstrom & Robertson 2005). Pollination by insects is completed by some of the 41 Hymenoptera (Donovan 2007), ~2000 Diptera, less than 30 butterflies, over 1800 moths and a few other invertebrates (Crowe 2002; Newstrom & Robertson 2005; Kelly *et al.* 2006). The Hymenopteran order covers bees, ants, and wasps while the Dipteran order covers flies, identified by their single set of wings (Crowe 2002). The exact number of pollinating species is not known (Newstrom & Robertson 2005), and below is a brief summary of some of the most important taxa.

Some of the most important pollinating insects in New Zealand are the bees (Hymenoptera). A large proportion of New Zealand's native bees are endemic, and 18 of these indigenous bee species can be considered relatively small and plain compared to their exotic counterparts (Donovan 1980; Newstrom & Robertson 2005; Donovan 2007). Only a few of New Zealand's native bees are social and New Zealand's fauna lacks large native social bees (Newstrom & Robertson 2005). This small size may reduce their pollination effectiveness. All of New Zealand's native bees belong to either the Colletidae (Newstrom & Robertson 2005; Donovan 2007) or Halictidae (Donovan 1980, 2007) and so far both groups are competing successfully with exotic insects and surviving introduced predators (Donovan 2007).

Another significant group of pollinators are the flies (Diptera). Of the Diptera in New Zealand, Tachinidae, Syrphidae, Tabanidae, Bibionidae, and Calliphoridae are all known to be important pollinating fly families (Newstrom & Robertson 2005). Flies may at times be more important in pollination than bees because of their capacity to pollinate in cold rainy weather (Newstrom & Robertson 2005). This is important for a number of reasons: New Zealand is known for its sporadic weather patterns, most of New Zealand's bee fauna is inactive over winter, and a significant portion of New Zealand's landscape is alpine (Newstrom & Robertson 2005).

Lepidoptera (i.e. butterflies and moths) are also active in alpine environments. Of the 13 native butterflies, 11 are endemic to New Zealand but no specialised pollinating systems have been found involving these species (Newstrom & Robertson 2005). There are over 1700 moths in New Zealand (Crowe 2002) and a number of these moth species are thought important pollinators. Some moths are active during the day (Newstrom & Robertson 2005) and therefore may be present during my observations. It is important to consider both native and exotic species as both are thought to contribute to successful pollination in New Zealand.

New Zealand has a number of exotic invertebrate species which may play an important role in supplementing pollination services weakened through species losses or declines (Kelly *et al.* 2006). Of the exotic bee pollinators, the majority were deliberately introduced for agricultural pollination (Donovan 2007; Howlett & Donovan 2010). Exotic Hymenoptera which are established in New Zealand include the honeybee (*Apis mellifera),* four species of bumblebee (*Bombus terrestris, Bombus hortorum, Bombus ruderatus,* and *Bombus subterraneus*), Lucerne leafcutting bee (*Megachile rotundata),* alkali bee *(Nomia melanderi),* red clover mason bee *(Osmia coerulescens)*, and the German and common wasp (*Vespula germanica* and *Vespula vulgaris*) (Crowe 2002; Newstrom & Robertson 2005; Kelly *et al.* 2006; Donovan 2007; Brockerhoff *et al.* 2010; Howlett & Donovan 2010; Webber *et al.* 2012) (for a general overview of NZ insects see Crowe 2002). Some exotic Diptera species such as the drone fly (*Eristalis tenax)* and green blowfly (*Lucilia sericata)*, as well as the monarch butterfly (*Danaus plexippus)*, and cabbage white (*Rapae pieris*) (Lepidoptera) are also relatively common in urban environments (Webber *et al.* 2012). The four introduced bumblebee species illustrate how different exotic species may exploit different niches in New Zealand. Only one (*Bombus terrestris*) is comparatively short-tongued for a bumblebee, while the remaining three less common species (*Bombus hortorum*, *Bombus ruderatus*, *subterraneus*) are long-tongued and morphologically adapted to different resources (i.e. flowers with long corolla tubes) (Howlett & Donovan 2010). *Apis mellifera* is widespread throughout New Zealand, while *Bombus* species have more restricted ranges (Howlett & Donovan 2010). The impact of *Apis mellifera* on the native flora of New Zealand is not fully understood and little investigation has been undertaken into the impact of exotic bee fauna on native bees (Howlett & Donovan 2010). Exotic bees likely have different preferences to the native bees because they have co-adapted alongside a different suite of plant species, and this may both reduce competition between native and exotic insects and increase the success of exotic plants.

The preferences of New Zealand's pollinating fauna

Campbell *et al.* (2010) recently addressed the lack of empirical evidence surrounding the preferences of New Zealand's native pollinators by looking at petal colour, flower morphology, and

familiarity of plant species chosen by native pollinating insects in an alpine environment. Insect visitors showed a range of preferences. Before flowers were manipulated *Hylaeus matamoko* preferred *Ourisia glandulosa* (white, gullet shaped) over *Ranunculus gracilipes* (yellow, dish-shaped), muscid flies (*Spilogona*) preferred *Ranunculus gracilipes* over *Ourisia glandulosa*, both *Allograpta* and *Leioproctus* species preferred *Brachyglottis bellidioides* (yellow rays) over *Brachyscome sinclarii* (white rays), and finally both *Allograpta* and *Platycheirus* species preferred *Ourisia caespitosa* (white, gullet-shaped) over *Ourisia glandulosa* despite the great similarities between the two species (*Ourisia caespitosa* has a larger yellow spot in its throat). Following painting of the flower petals, the preferences of insect species showed a trend towards the colour most closely matching the species they had preferred in the previous experiment. This was illustrated by *Allograpta* which preferred yellow over white flowers regardless of whether *Ranunculus gracilipes* or *Ourisia glandulosa* was painted yellow. In sum, naturally yellow flowers painted yellow received significantly more visits. However, naturally white plants painted white were not similarly favoured (Campbell *et al.* 2010). Finally arrays were set up which attempted to determine the effect of other traits on the colour preferences of flower visiting invertebrates. This saw a significant interaction between flower species and the response of pollinators to colour, with *Brachyglottis bellidioides* painted yellow receiving the most visits from native bees and the flies, *Allograpta* and *Platycheirus* species Morphological manipulations which lead to a flower more closely resembling a preferred species were also often significantly preferred by visitors. These results suggest that flower visiting insects may more often prefer familiar suites of traits, perhaps learned through frequent visitation to particular species. My chapter will extend Campbell *et al.*'s (2010) findings into an urban environment to determine whether flower visiting invertebrate species also show colour preferences dependent upon flower provenance.

Plant-pollinator provenance in New Zealand

In New Zealand, introduced hymenoptera appear to have penetrated native systems better than the introduced avifauna (Kelly *et al.* 2006). One review which attempted to quantify the role of exotic invertebrates in New Zealand's pollination services based on the frequency of pollinator visitation found that exotics made up more than half of the total visits in 5 out of the 15 cases included across 12 plant species (Kelly *et al.* 2006). Interestingly, only 6 of these plant species conformed to an entomophilous (insect-pollinated) syndrome, and were visited less often on average by the introduced hymenoptera than the remaining 6 species. In their review 29% of all visits to flowers were by the introduced Hymenoptera, and of these *Vespula* or *Bombus* accounted for the majority of visits despite *Apis mellifera*'s greater numbers (Kelly *et al.* 2006). All of the four *Bombus* species found in New Zealand have been shown to prefer exotic over native flowers, and particularly exotics of European origin (Goulson & Hanley 2004). Goulson and Hanley (2004) recorded only one of these species, *Bombus terrestris*, visiting native flowers, although some other species have been infrequently seen to visit natives (Donovan 2007). In New Zealand, *Bombus terrestris* has been recorded visiting 419 plants. Of these, 19 were native and 400 were exotic (Goulson 2003). Hanley and Goulson (2003) surmised that introduced pollinators tend to prefer introduced flowers in New Zealand. They also found a positive correlation between the presence of introduced bee species and the spread of weeds in New Zealand. Because of the significant exotic visitation mentioned, further investigations may wish to determine whether the preferences of these exotic invertebrate pollinators may change the direction of New Zealand's floral evolution.

Resistance to invasions and the facilitation of weeds

The impact of foreign mammals and plants upon New Zealand's ecosystems has been well documented, however there is still much to be learnt surrounding the impacts of invading invertebrates (Brockerhoff *et al.* 2010; Howlett & Donovan 2010). The preferences of foraging invertebrate species may lead to changes in the composition of New Zealand's flora (Howlett & Donovan 2010). Introduced bees are thought to prefer to forage on introduced plants, and these introduced plants have been shown in a New Zealand study to exhibit an increased seed set when visited by non-native bee species. Additionally, native plants may receive low quality pollination such as a lack of pollen transfer or nectar robbing (Hanley & Goulson 2003). Four weed species in particular (*Berberis darwinii, Lupinus arboreus, Ulex europeaus,* and *Cytisus scoparius),* are dependent upon *Apis mellifera* and *Bombus* species for pollination (Goulson 2003; Howlett & Donovan 2010) and, as mentioned in the previous paragraph, a positive relationship between the presence of exotic bee species and the proliferation of weeds exists (Hanley & Goulson 2003). Conversely, while *Apis mellifera* visits many weed species in New Zealand (~43% of weeds), these weed species employ plastic reproductive strategies (i.e. selfing or low specialisation). These strategies reduce their dependency upon pollinators and thereby reduce the likelihood that *Apis mellifera* increases the reproductive success of weeds (Butz Huryn 1995). Brockerhoff *et al.* (2010) assert that the lack of documented impacts of invertebrates upon New Zealand's native species and ecosystems is the result of an 'inherently resistant' ecosystem. Much knowledge is still needed to support this theory because of a bias towards economically important species in the literature (Brockerhoff *et al.* 2010).

Campbell *et al.*'s (2010) work supports Brockerhoff *et al.*'s (2010) hypothesis as when native insect pollinator preferences were detected they were for familiar morphs. However later work by Campbell *et al.* (2012) found that native bee species would visit white *Wahlenbergia albomarginata* flowers painted blue when experiments were set up at a large experimental scale. Pollinators are probably not driving the change to pale colouration of this flower species. Therefore native pollinators may visit plants which do not express the traits common to the native flora. A major limitation of previous work by Campbell *et al.* (2010; 2012) is the restriction to relatively undisturbed alpine habitat. My chapter can contribute to Brockerhoff *et al.*'s assertion by extending the framework into the Christchurch city and testing whether exotic bee species are equally abundant across different communities. I also test whether Campbell *et al.*'s results (that native bees sometimes show preferences for colours other than white and this can depend upon a number of other factors) apply to urban environments. Urban areas are particularly worth of consideration because of the continued expansion of human settlements and land clearance for development.

Chapter objectives

Specifically I determine whether there is a significant difference in the abundance of insect pollinators depending on their provenance (native versus exotic) and the provenance of the most abundant plants in the wider community at a site. I use three sites of increasing urbanisation; the Sign of the Bellbird in the Port Hills, the Christchurch Botanical Gardens, and the University of Canterbury; where flowering exotic angiosperms are abundant. I hypothesize that native invertebrate pollinators are more abundant in areas dominated by native plants, while exotic

pollinators are more common in areas of predominantly introduced flora. If the abundance of the pollinating insect fauna correlates with the level of urbanisation of the site, then it will be useful to determine whether this is through a preference for flowers of matching provenance, the colouration of the flower, or an interaction between these factors. Testing these ideas will provide greater understanding of New Zealand's unique pollinating system. From here conservation efforts targeting particular aspects of behaviour or preference may be refined to be more successful, and a better understanding of the relationship between New Zealand plants and their visitors may be gained.

Specifically, the following questions are addressed:

1. Is the proportion of natives relative to exotic pollinators higher in native-plant-dominated communities?
2. Do native insects prefer native flowers, while introduced insect pollinators preferentially visit exotic flowers or lack preference?
3. Does flower colour predict pollinator visitation under natural conditions?
4. Do these data support Brockerhoff et al's (2010) assertion that the New Zealand flora is inherently resistant to faunal invasion?

These questions are tested by observing the insect visitors to a large range of native and exotic flowers. The wider Christchurch area presents an excellent system to test the relationship between plant-pollinator provenances. This is because Christchurch has a gradient of urbanisation from highly urban educational facilities and parks to sites containing secondary native forest. By expanding Campbell *et al.*'s (2010; 2012) results into different systems trends across New Zealand may be identified. All flying insects resting upon a flower will be counted, and are hereby referred to as flower visitors. This is because determining whether pollen collection and deposition occurred is beyond the scope of this study.

2.2 Methods

Study sites and species

I used these study sites: Christchurch Botanical Gardens (43.53°S, 172.62°E), areas near the Sign of the Bellbird on the Port Hills (43.63°S, 172.61°E), and the University of Canterbury (43.53°S, 172.68°E). The Christchurch Botanical Gardens, University of Canterbury, and the Port Hills were chosen because they represent a gradient of human interference. The University of Canterbury is exotic-plant dominated at the community level, with a mix of native and exotic plants in their gardens. The Botanic Gardens cover 21 ha of land, with a native plant section within a wider exotic plant landscape. The native plant section covers land from the Water Garden to the Avon River in the south, and to Berwick's Walk in the east. Exotic plants observed were located in the Herbaceous Border which is 159 m long by 6.4 m wide (Christchurch Botanic Gardens Management Plan, http://resources.ccc.govt.nz/files/ChChBotanicGardensMgtPlan2007_ALL-christchurchbotanicgardens.pdf, date accessed 12 September 2013). Land in the Port Hills consists of a variety of land uses such as farmland, housing, and bush. It is been undergoing restoration efforts since the early 1900s (Orwin 2008). The Port Hills also incorporate a number of suburbs considered a

part of Christchurch City; Cashmere, Healthcote, and Sumner; as well as sections of farming and forestry (Orwin 2008). The particular Port Hills sites chosen are therefore native-plant dominated with less human interaction, while still open to the public. These Port Hills sites consisted of secondary native forest and two open grassland areas within a wider grass and forest landscape.

Plant taxa were picked from those flowering at each sampling event. Plant taxa observed varied as some plants finished flowering early or came into flower later in the season. Species from 14 plant families were used in this study (Table 2.1). Attempts were made to maintain relatively equal numbers of native and exotic plant species at each site; however this was restricted depending on the seasonality of the taxa. Plant species which finished flowering before they could be observed more than five times were omitted from results and 14 plant species had only one plant observed at a site. All winged invertebrates found to be resting on flowers were included as a flower visitor. No flower visitors (even those not thought to provide a pollination service) were excluded from results except for a few rare visitors that were unable to be identified; therefore the data include a range of bees, flies, wasps, butterflies, grasshoppers, and beetles.

Insect observations

Data collection ran 19 January 2012 until 13 April 2012, and consisted of 28 days of sampling. Collection began in the Botanic Gardens on 19 January 2012, before moving to include the University of Canterbury on 22 January 2012, and lastly the Port Hills on 29 February 2012. Observations were conducted 35 times over this period as repeats were sometimes taken on the same day. Data collection on the Port Hills started in February as a result of the later flowering times at this site. The weather during this period was particularly cool and wet for February (Table 2.1). To maximise insect observations across species I prioritized days with warm weather. As a result sampling intervals were irregular. I defined a visitor as any insect sitting upon a given flower(s) at the time I approached it. Samples were instantaneous and were taken during daylight hours on fine days after 10am but before 5pm. Instantaneous sampling here was defined as counting all of the insects on the flowers of a focal plant at approximately one instance. I observed frequent swarms of native *Lasioglossum* or *Hylaeus* species flying around plants, but significantly less individuals actually on the flowers at the Christchurch Botanical Gardens. Unknown species were identified using Crowe (2002), Parkinson (2007), and Donovan (1980).

Environmental data

In order to include environmental variation I collected the following data: site, date, month, time, temperature (°C), sun shade, wind (ms^{-1}) and cloud cover at each set of visits. If more than one plant individual was observed during a sampling event then this was given a unique identification code, and flower colour was also recorded. Data were later supplemented with plant and insect family information. Plants that had no visitors were included as zeroes to measure preference (as choosing not to visit a flower is a preference). Environmental conditions were recorded as follows; cloud cover was estimated as a fraction of eight of the visible sky when I was standing in front of the focal plant. Sun shade was recorded on an ordinal scale from 1-6 (open, spotted, patchy, light shade, shade, and deep shade) on the observed plant. Air temperature was recorded using a QUARTZ digi-thermo thermometer in °C under shade, and wind speed was taken using a hand held Deuta Anemo wind meter in units of ms^{-1} at a height of approximately 1.70 m.

Table 2.1: Mean rainfall (mm) and temperature (°C) from January-April over three consecutive years (The National Climate Database, http://cliflo.niwa.co.nz/, date accessed 11 August 2013). 1mm+ represents the number of days for that month in which more than 1mm of rain fell, while 0.1mm+ represents the number of days that 0.1+ mm of rain fell.

Rainfall	**2011**			**2012**			**2013**		
	Total	1mm+	0.1mm+	Total	1mm+	0.1mm+	Total	1mm+	0.1mm+
January	55.6	6	12	39.0	6	8	34.0	4	6
February	36.6	4	10	48.2	6	10	20.4	1	2
March	49.0	8	13	38.8	6	9	22.0	2	5
April	67.2	7	10	34.6	3	7	72.6	6	9
Temperature	**2011**			**2012**			**2013**		
	Mean	Highest	Lowest	Mean	Highest	Lowest	Mean	Highest	Lowest
January	18.1	22.8	13.4	16.6	21.8	11.3	18.4	24.4	12.5
February	18.1	22.6	13.6	16.1	20.0	12.3	17.3	22.7	11.9
March	16.0	20.6	11.5	14.0	18.9	9.1	16.6	21.9	11.4
April	12.1	16.5	7.7	12.4	17.8	7.1	12.9	18.0	7.9

Statistical analysis

I carried out analysis at three levels of increasing detail: plant provenance, plant species and individual plant.

General trends of plant-insect provenance (chi-square tests)

To test the null hypotheses: the proportion of native insects relative to exotic insects is not higher in native-plant-dominated habitats a chi-square test was run in Microsoft Excel (version 14.0). This was done by comparing the total number of native and exotic insects at the three sites (i.e. Port Hills, University of Canterbury, and Botanical Gardens).

Following this chi-square test, data were also combined to test the hypothesis: native insects prefer native flowers, while introduced insect pollinators preferentially visit exotic flowers. A chi-squares test (in a two by two square of plant provenance against insect provenance) was run by totalling the number of native and exotic insects on native and exotic plants and comparing the expected versus observed values.

Finally, a chi-square test was also used to determine whether floral traits predict pollinator visitation. Here, the total numbers of native and exotic insects were tested against white and not-white native and exotic plants in a 2x4 table (i.e. plants were divided into four classes: native white, native not-white, exotic white, and exotic not-white). This was also run in Microsoft Excel (version 14.0).

Plant species level analysis (GLM)

In this section trends of all native and exotic insects observed were analysed using generalised linear models (GLM) with the "lme4" package from R (version 2.15.0, R Foundation for Statistical Computing 2012). This model (preference.glm below) allowed me to determine preference of flower visiting insects by looking at the proportions of visits to certain plant species against all visits by that insect species at a site. Hence the level of replication was individual plant species (n = 39 species).

Because data are overdispersed the quasibinomial family was used for this model. There were sufficient numbers of purple, white, and yellow flowers to test these separately, and all remaining colours were pooled into an 'other' category.

Firstly the model was reordered so that all colours were tested against white flowers:

```
mid$colour<-relevel (mid$colour, ref="white")

preference.glm <- glm (cbind (totalperplantspp, otherpersite) ~ insecttype
* planttype + colour + site + insecttype:colour, family = quasibinomial,
data = mid)
anova (preference.glm, test="F")
summary (preference.glm)
```

Plant-level analysis (GLMM)

In this final section the preferences of insect species for flower provenance and colour were analysed, with replication at the individual plant level (n=2068). Sampling yielded sufficient insect visitation counts to analyse the seven most common insect species across plant taxa with white, purple, or yellow flowers. The remaining colours were not included in this analysis because of lack of visitation. Those insect taxa common enough for further analysis were the two exotic bees; *Apis mellifera* and *Bombus*, the exotic syrphid fly *Eristalis tenax,* the native bee: *Lasioglossum, Hylaeus,* and *Leioproctus*, and the native syrphid fly *Melangyna novaezelandiae*. These insects were tested for preferences in regards to the provenance of the plant and the colour of the flower. Because the original data file lacked zero counts (i.e. if an insect was not observed on a flower then no data for that insect was recorded) the data were first reshaped in R to include all zeroes using the "reshape" package. Therefore every plant species then had data on each of the possible seven insects that could have visited.

Because my response variable was the total number of the insect species (in the case below: *Bombus*) counted on the flowers of a focal plant in a single instance (count data), a poisson error distribution was used, and a nested error term (individual plant within a plant species at a particular site) was included which accounted for the nested data structure between and within sites, as well as within plant species.

To further test the first three hypotheses: the proportion of natives relative to exotics is higher in native-plant-dominated habitats; native insects prefer native flowers, while introduced insect pollinators preferentially visit exotic flowers; and floral colour predicts pollinator visitation; I used a poisson generalised linear mixed model (GLMM) with the "lme4" package from R (version 2.15.0, R Foundation for Statistical Computing 2012). GLMMs were chosen because of the non-normality of the error distribution and the ability to account for plant-level effects as a nested random term. Using this method, I also tested for the effects of additional covariates including temperature, sun shade, cloud, site, nested replication, and wind.

Therefore, the R code for the data reshape was:

```
wide.final = reshape (data = feb [,c(1:16)], v.names = "Numberseen",
idvar=c ("ID","Plantspp", "Planttype", "Family", "Colour", "Insectspp",
"Date", "Month", "Daterep",  "Wind", "Temp", "Exposure", "Cloud", "Site",
"WYP"), timevar = "Insectspp", direction="wide")

wide.final[,14:22][is.na(wide.final[,14:22])] <- 0

dat1 <- (melt (wide.final, id=names (wide.final) [1:14]) )
dat2 <- cast (dat1, ID + Plantspp + Planttype + Family + Colour + Date +
Month + Daterep + Wind + Temp + Exposure + Cloud + Site + WYP ~ variable,
sum)
```

I used model selection based on likelihood ratio tests in R to test among different models with and without the inclusion of different environmental conditions, nested random terms, and fixed effects (i.e. 'anova(model1,model2)' command). This resulted in the exclusion of cloud and wind, and a nested error term which included site, plant species, and the plant observed.

For example, the R code for *Bombus* was:

```
Bombus.model <-glmer (Numberseen.Bombus ~ Planttype + as.factor (WYP) +
Temp + Shade + (1|Site/Plantspp/ID), data=dat2, family=poisson)
```

The predictors included in the model (above) were the provenance of the plant (Planttype), the air temperature in shade (Temp), the level of sun shade of a plant (Shade), and the colour of the flower petals (WYP). While ideally the interaction between some of these predictors would have been interesting, this was unable to be tested for because of limited data.

2.3 Results

My observations detected a total of 38 different insect taxa (18 introduced and 20 native (Table 2.2)) over 39 plant taxa (25 introduced, 14 native (Table 2.3)). Each plant was observed as a snapshot, with total insects on flowers counted in one instance in an attempt to reduce the likelihood of counting the same insect twice. The most common exotic visitors were *Bombu*s species, *Apis mellifera,* and *Eristalis tenax*; while the most common native visitors were *Hylaeus* species, *Lasioglossum* species, *Leioproctus* species, and *Melangyna novaezelandiae*. The remaining taxa made less than 50 total visits each and those with less than 5 visits counted were excluded from analysis. A large proportion of the total visitation (~89%) was performed by seven insect taxa. Clear trends across plant origins were not detected (Table 2.3).

Table 2.2: The number of exotic (top) and native (bottom) insect flower visitors observed in this study and their percentage of the total visitation. Counts from the Christchurch Botanical Gardens, University of Canterbury, and the Port Hills are combined.

Type	Order	Family	Insect taxon	%	Total	Origin
Exotic	Diptera	Calliphoridae	*Calliphora stygia*	0.10	4	Australia
	Diptera	Calliphoridae	*Calliphora vicina*	0.13	5	Europe
	Diptera	Calliphoridae	*Lucilia sericata*	0.60	23	Europe
	Diptera	Drosophilidae	*Drosophila* species	1.19	46	Worldwide
	Diptera	Fanniidae	*Fannia canicularis*	0.05	2	Worldwide
	Diptera	Muscidae	*Musca domestica*	0.18	7	Worldwide
	Diptera	Muscidae	*Muscina stabulans*	0.36	14	Worldwide
	Diptera	Sarcophagidae	*Oxysarcodexia varia*	0.49	19	South America
	Diptera	Syrphidae	*Eristalis tenax*	6.46	249	Europe
	Diptera	Syrphidae	*Merodon equestris*	0.03	1	Europe
	Hymenoptera	Apidae	*Apis mellifera*	27.44	1058	Europe, Asia & Africa
	Hymenoptera	Apidae	*Bombus* species	27.54	1062	Europe
	Hymenoptera	Megachilidae	*Anthidium manicatum*	0.31	12	Europe, Asia & Africa
	Hymenoptera	Vespidae	*Ancistrocerus gazella*	0.54	21	Europe
	Hymenoptera	Vespidae	*Vespula germanica*	0.86	33	Europe, Asia & Africa
	Hymenoptera	Vespidae	*Vespula vulgaris*	0.16	6	Europe & Asia
	Lepidoptera	Nymphalidae	*Danaus plexippus*	0.13	5	North America
	Lepidoptera	Pieridae	*Pieris rapae*	0.78	30	Europe, Asia & Africa
				67.35	**2597**	
Native	Coleoptera	Cerambycidae	*Zorion* species	0.05	2	New Zealand
	Diptera	Asilidae	*Neoitamus melanopogon*	0.13	5	New Zealand
	Diptera	Calliphoridae	*Calliphora quadrimaculata*	0.08	3	New Zealand
	Diptera	Chironomidae	*Chironomus zealandicus*	0.05	2	New Zealand
	Diptera	Dolichopodidae	*Parentia* species	0.36	14	New Zealand
	Diptera	Simuliidae	*Austrosimulium*	0.47	18	New Zealand
	Diptera	Stratiomyidae	*Beris* species	0.03	1	New Zealand
	Diptera	Syrphidae	*Helophilus hochstetteri*	0.44	17	New Zealand
	Diptera	Syrphidae	*Melangyna novaezelandiae*	2.10	81	New Zealand
	Diptera	Syrphidae	*Melanostoma fasciatum*	0.86	33	New Zealand
	Diptera	Tabanidae	*Scaptia adrel*	0.03	1	New Zealand
	Diptera	Tipulidae	*Leptotarsus* species	0.05	2	New Zealand
	Hymenoptera	Colletidae	*Hylaeus* species	13.15	507	New Zealand
	Hymenoptera	Colletidae	*Leioproctus* species	3.32	128	New Zealand
	Hymenoptera	Halictidae	*Lasioglossum* species	9.10	351	New Zealand
	Lepidoptera	Lycaenidae	*Lycaena salustius*	0.78	30	New Zealand
	Lepidoptera	Nymphalidae	*Vanessa gonerilla gonerilla*	1.27	49	New Zealand
	Lepidoptera	Nymphalidae	*Vanessa itea*	0.21	8	New Zealand
	Odonata	Coenagrionidae	*Xanthocnemis zelandica*	0.03	1	New Zealand
	Orthoptera	Tettigoniidae	*Conocephalus* species	0.16	6	New Zealand
				32.65	**1259**	
Grand total				**100%**	**3856**	

Table 2.3: Total number of insect visits to introduced (top) and native (bottom) plants. Counts from the Christchurch Botanical Gardens, University of Canterbury, and the Port Hills are combined.

Plant type	Family	Plant taxa	Colour	%	Total	Origin
Introduced	Araliaceae	*Acanthopanax lasiogyne*	Green	1.48	57	China
	Amaryllidaceae	*Agapanthus* species	Purple	0.67	26	South Africa
	Iridaceae	*Crocosmia* species	Red	0.60	23	South Africa
	Asteraceae	*Achillea credo*	Yellow	2.05	79	Europe, Asia, & North America
	Asteraceae	*Achillea millefolium*	White	9.34	360	Europe, Asia, & North America
	Asteraceae	*Argyranthemum frutescens*	White	0.62	24	Canary Islands
	Asteraceae	*Artemisia lactiflora*	White	0.41	16	Western China
	Asteraceae	*Aster amellus*	Purple	4.15	160	Europe & Asia
	Asteraceae	*Bellis perennis*	White	0.13	5	Europe
	Asteraceae	*Crepis capillaris*	Yellow	0.29	11	Europe
	Asteraceae	*Helenium flexuosum*	Yellow	10.48	404	North America
	Asteraceae	*Hypochaeris radicata*	Yellow	2.41	93	Europe
	Asteraceae	*Inula hookeri*	Yellow	1.12	43	Europe, Asia, & Africa
	Asteraceae	*Taraxacum officinale*	Yellow	0.23	9	Europe & Asia
	Asteraceae	*Aster* species	Yellow	0.54	21	Europe & Asia
	Caprifoliaceae	*Leycesteria formosa*	White	0.57	22	Himalaya & China
	Fabaceae	*Trifolium repens*	White	0.16	6	Europe, Africa, & Asia
	Lamiaceae	*Agastache* 'Blue Fortune'	Purple	4.67	180	Asia & North America
	Lamiaceae	*Monarda* species	Red	3.27	126	North America
	Lamiaceae	*Salvia guaranitica*	Purple	5.39	208	South America
	Lamiaceae	*Salvia verticillata*	Purple	1.53	59	Europe & Asia
	Lamiaceae	*Stachys cretica* sub *cassia*	Purple	6.64	256	Europe & Asia
	Onagraceae	*Fuchsia* species	Purple	1.09	42	America, NZ, Tahiti & Hispaniola
	Rosaceae	*Rubus fruticosus*	Pink	0.41	16	Europe, Africa, Asia, & America
	Convolvulaceae	*Calystegia soldanella*	White	1.14	44	North America
				59.39	**2290**	
Native	Asteraceae	*Brachyglottis cultivar*	Yellow	0.21	8	New Zealand
	Asteraceae	*Brachyglottis greyi*	Yellow	0.05	2	New Zealand
	Asteraceae	*Brachyglottis saxifragoides*	Yellow	0.16	6	New Zealand
	Asteraceae	*Olearia avicenniifolia*	White	6.66	257	New Zealand
	Asteraceae	*Pachystegia insignis*	White	1.06	41	New Zealand
	Campanulaceae	*Pratia angulata*	White	0.44	17	New Zealand
	Polygonaceae	*Muehlenbeckia astonii*	White	9.15	353	New Zealand
	Fabaceae	*Carmichaelia* species	Purple	0.23	9	New Zealand
	Plantaginaceae	*Hebe franciscana*	Purple	4.49	173	New Zealand
	Plantaginaceae	*Hebe salicifolia*	White	2.59	100	New Zealand
	Plantaginaceae	*Hebe strictissima*	White	11.00	424	New Zealand
	Plantaginaceae	*Hebe Wiri Joy*	Purple	2.39	92	New Zealand
	Plantaginaceae	*Hebe Wiri Mist*	White	2.15	83	New Zealand
	Myrtaceae	*Lophomyrtus x ralphii*	White	0.03	1	New Zealand
				40.61	**1566**	
Grand total				**100%**	**3856**	

There was a greater proportion of exotic plant taxa observed at both species and plant level across all sites surveyed (Table 2.4). I attempted to keep the number of native and exotic plants observed equal, which was roughly successful at the Botanic Gardens and University of Canterbury, but not so at the Port Hills sites.

Table 2.4: The total number of plant individuals and plant species sampled at each site

Site	Total plant individuals		% of plants observed		Total plant species	
	Native	Introduced	Native	Introduced	Native	Introduced
Botanic Gardens	22	28	44	56	8	15
Port Hills	23	117	16	84	4	8
University of Canterbury	18	23	44	56	2	2

Chi-squares tests

Trends across sites

Because of the differences in the number of plants observed, an early analysis using a chi-square test was run to determine whether there was a significant difference in the proportion of visits by native and exotic insects at different sites. This was significant (Table 2.5, X^2= 259.98, P<0.0001, 2df), with the lowest proportion of native visitors observed at the Botanic Gardens, and the highest proportion at the Port Hills. Insect abundances were consistent with the hypothesis that native insect taxa will be relatively more abundant than exotic pollinating species in areas dominated by native plants at the community level (as the Port Hills is an area of secondary native forest), while exotic plant visitors are more abundant in areas of predominantly exotic plant taxa.

Table 2.5: The counts of exotic and native pollinators observed at the three different sites.

Site	Introduced insects	Native insects	% of insects native
Botanic Gardens	1801	556	23.6
University of Canterbury	220	112	33.7
Port Hills	576	591	50.6

Trends in visitation preferences across all insect taxa

A broad scale analysis of all insect taxa was run to determine whether native pollinators prefer native plants and exotic pollinators prefer exotic plants. This chi-square two-row-by-two-column test (native and exotic insects against native and exotic plants) was consistent with the hypothesis that native insect visitors will preferentially visit native plants, while introduced insect taxa will prefer exotic plants (X^2=446.54, P<0.001, 1df); both exotic and native insect taxa are less likely to visit native and exotic plants respectively (Table 2.6).

Table 2.6: The total observed number of native and introduced insects on native and introduced plants (with expected values in brackets).

	Introduced plants	**Native plants**
Introduced insects	1845 (1542)	752 (1055)
Native insects	445 (748)	814 (511)
% of insects native	19.4	52.0

Including broad colour categories (white or not-white) and the provenance of the plant (native or exotic) in another chi-squares analysis determined that native visitors showed a strong preference for white flowers; particularly those of native origin, while exotic pollinators showed a strong preference for exotic flowers that were colours other than white (X^2= 864.3, P<0.0001, 3df) (Table 2.7). Therefore native insect taxa visit native white flowers more often and exotic taxa prefer exotic coloured flowers.

Table 2.7: The observed and expected (in brackets) number of flower visitors depending on their own provenance and both the colour and provenance of the plant observed. The figures in bold have the largest deviance from the expected value for native and exotic insects.

	Plants			
	Colours other than white		**White**	
	Introduced	**Native**	**Introduced**	**Native**
Introduced insects	**1623** (1221.05)	211 (195.31)	222 (321.26)	541 (859.38)
Native insects	190 (591.95)	79 (94.69)	255 (155.74)	**735** (416.62)

GLM plant species level analysis

Trends in the proportion of visits to plants for all insects using GLM

I then used a quasibinomial GLM analysis to account for variation across sites by looking at the proportion of visits to a plant species out of the sum of that insect's visits at that site (preference.glm in Methods section above). The interaction between insect and plant provenance, and the interaction between insect provenance and flower colour, was significant (Table 2.8-2.9). Native insects showed a significant preference both for native plants (P<0.001, t=3.59), and for white over both purple (P<0.001, t=-3.91) and yellow flowers (P=0.002, t=-3.17).

Table 2.8: Parameter estimates, standard error, z statistics, and *P*-values of a quasibinomial GLM for all (38) insect taxa, testing the effects of site, plant, colour, and insect provenance, and their interaction. Significant effects are highlighted in bold.

Fixed effects	Estimate	Std. Error	t value	Pr (>|t|)
(Intercept)	-3.11	0.20	-15.78	<0.001
Insect type (native)	0.21	0.29	0.71	0.48
Plant type (native)	-0.41	0.20	-2.09	**0.04**
Colour (other)	-0.26	0.34	-0.76	0.45
Colour (purple)	0.50	0.22	2.30	**0.02**
Colour(yellow)	-0.03	0.25	-0.13	0.90
Site (PH)	0.67	0.18	3.69	**<0.001**
Site (UC)	0.69	0.26	2.71	**0.007**
Insecttype(native):Planttype(native)	1.17	0.33	3.59	**<0.001**
Insecttype(native):Colour(other)	-2.82	1.60	-1.76	0.08
Insecttype(native):Colour(purple)	-1.79	0.46	-3.91	**<0.001**
Insecttype(native):Colour(yellow)	-1.46	0.46	-3.17	**0.002**

Table 2.9: The effect of plant provenance, colour and site on the visitation (proportion of visits to plants out of totals at each site) of all (39) insect taxa at Christchurch Botanical Gardens, Port Hills, and the University of Canterbury, from the minimal adequate quasibinomial GLM. Significant effects are highlighted in bold.

	Df	Deviance	Resid. Df	Resid. Dev.	F	Pr(>F)
NULL			1019	11904		
Insecttype	1	16.47	1018	11887	0.98	0.32
Planttype	1	29.21	1017	11858	1.74	0.19
Colour	3	283.30	1014	11620	4.72	**0.003**
Site	2	299.50	1012	11320	8.91	**<0.001**
Insecttype: planttype	1	478.43	1011	10842	28.45	**<0.001**
Insecttype: colour	3	448.41	1008	10393	8.89	**<0.001**

GLMM plant-level analysis

Trends in visitation preferences for the seven most abundant insects

The seven most common insect visitors were analysed separately, consisting of five bees and a native and exotic syrphid fly. While *Leioproctus* species, and *Hylaeus* species all showed a significant preference for native flowers over exotic plant species (Table 2.10-2.11), no insects showed a preference for exotics. This is surprising considering earlier results which showed a correlation between plant and pollinator provenance.

For three of the seven taxa, floral colouration (white, yellow, or purple) was a significant predictor of visitation (Table 2.10-2.11) with *Apis mellifera* and *Bombus* species both showing a significant preference for purple over white flowers, and *Melangyna novaezelandiae* preferring white over yellow flowers. The interaction between plant provenance and colour was unable to be tested for when performing analysis at this level. However, the average visitation of *Apis mellifera* and *Bombus*

taxa to purple exotic flowers was highest per observation set, while the mean insects on native plants per observation for native insect visitors was generally higher regardless of colour (Fig. 2.1).

Table 2.10: Parameter estimates, standard error, z statistics, and *P*-values for all fixed effects for 7 minimal adequate poisson GLMMs; using white, yellow, and purple flowers only for 7 flower visiting insects. This model was testing the effect of plant provenance, flower colour, temperature and shade. Plant individuals nested within plant species within a site were included as a nested random effect and all significant effects are in bold.

Species	Fixed effects	Estimate	Std. Error	z value	Pr(>|z|)
Apis mellifera	(Intercept)	-3.05	0.80	-3.84	**<0.001**
	Plant type: Native	0.87	0.79	1.10	0.27
	WYP: Purple	3.25	0.87	3.72	**<0.001**
	WYP: Yellow	0.56	0.97	0.58	0.56
	Temperature	-0.03	0.01	-2.19	**0.03**
	Shade	-0.09	0.02	-4.97	**<0.001**
Bombus	(Intercept)	-3.54	0.91	-3.91	**<0.001**
	Plant type: Native	-0.20	0.94	-0.22	0.83
	WYP: Purple	4.18	1.02	4.10	**<0.001**
	WYP: Yellow	0.17	1.18	0.14	0.89
	Temperature	-0.05	0.01	-3.45	**<0.001**
	Shade	0.08	0.02	4.66	**<0.001**
Leioproctus	(Intercept)	5.42	1.34	-4.06	**<0.001**
	Plant type: Native	2.99	0.92	3.27	**0.001**
	WYP: Purple	0.50	1.08	-0.46	0.64
	WYP: Yellow	0.69	1.19	-0.58	0.56
	Temperature	0.01	0.04	-0.29	0.78
	Shade	0.22	0.05	-3.99	**<0.001**
Lasioglossum	(Intercept)	6.29	0.99	-6.39	**<0.001**
	Plant type: Native	1.34	0.74	1.82	0.07
	WYP: Purple	1.15	0.98	-1.18	0.24
	WYP: Yellow	-0.36	0.86	-0.42	0.68
	Temperature	0.13	0.03	5.02	**<0.001**
	Shade	0.21	0.04	5.32	**<0.001**
Hylaeus	(Intercept)	4.01	0.76	5.25	**<0.001**
	Plant type: Native	2.17	0.72	3.00	**0.003**
	WYP: Purple	0.20	0.88	0.23	0.82
	WYP: Yellow	-0.92	0.89	-1.04	0.30
	Temperature	0.001	0.02	0.07	0.95
	Shade	0.17	0.03	-6.11	**<0.001**
Eristalis tenax	(Intercept)	-4.18	1.14	-3.67	**<0.001**
	Plant type: Native	0.18	0.73	0.25	0.80
	WYP: Purple	-1.92	1.06	-1.82	0.07
	WYP: Yellow	0.88	0.79	1.12	0.26
	Temperature	0.01	0.03	0.38	0.71
	Shade	0.03	0.05	0.57	0.57
Melangyna novaezelandiae	(Intercept)	0.25	1.02	0.24	0.81
	Plant type: Native	0.65	0.60	1.08	0.28
	WYP: Purple	1.16	0.64	1.81	0.07
	WYP: Yellow	-1.78	0.91	-1.96	**0.05**
	Temperature	-0.18	0.05	-3.76	**<0.001**
	Shade	-0.43	0.09	-4.83	**<0.001**

Table 2.11: Summary of the direction of fixed effects (plant provenance, plant colour, temperature, and shade) on 7 insects where + denotes a positive significant relationship, - denotes a negative significant relationship, and NS represents a non-significant result; extracted from results in Table 2.10.

Insect	Native over exotic	Yellow over white	Purple over white	Temp	Shade
Apis mellifera	NS	NS	+	-	-
Bombus	NS	NS	+	-	+
Leioproctus	+	NS	NS	NS	+
Lasioglossum	NS	NS	NS	+	+
Hylaeus	+	NS	NS	NS	+
Eristalis tenax	NS	NS	NS	NS	NS
Melangyna novaezelandiae	NS	-	NS	-	-

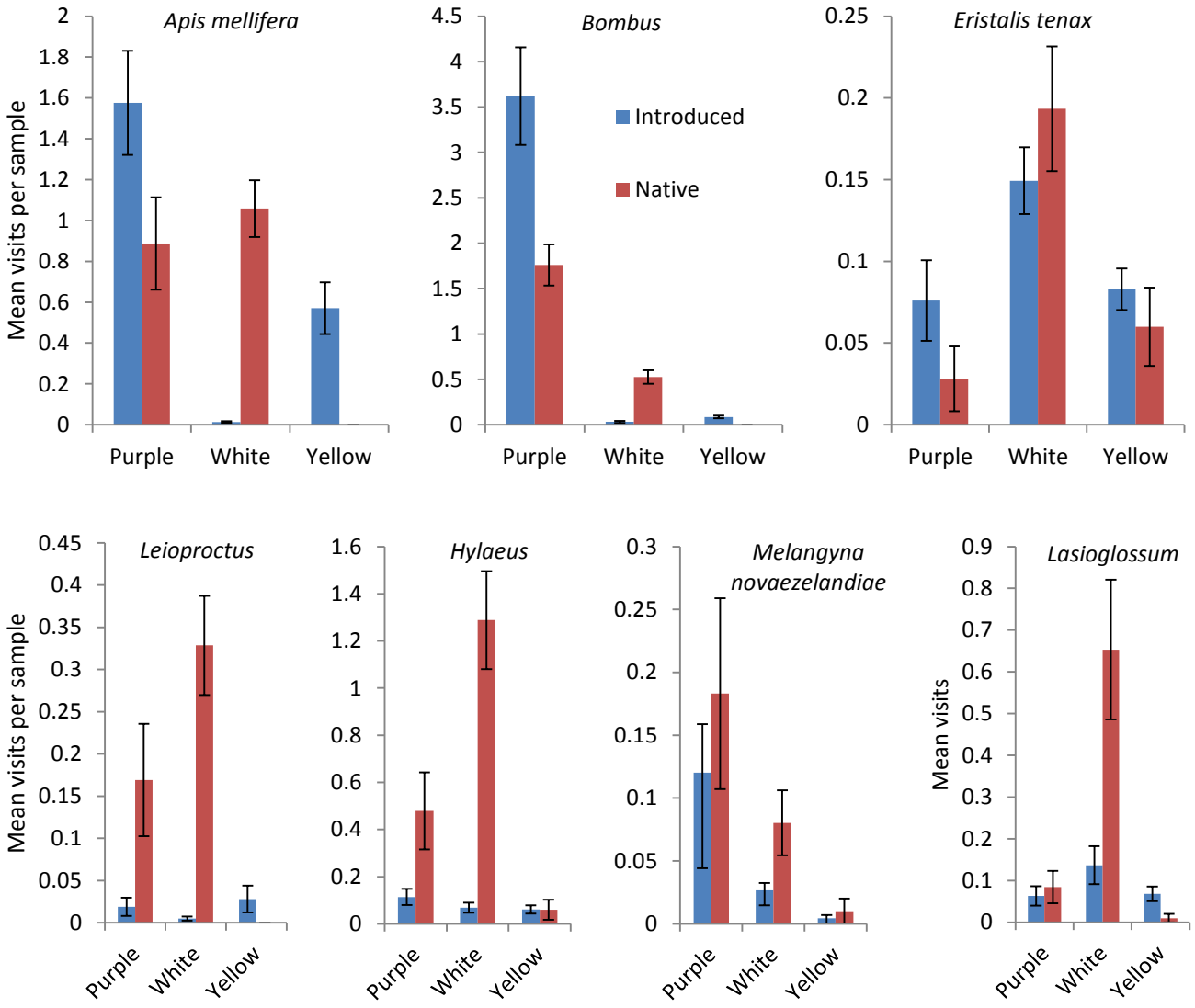

Figure 2.1: Mean number of visits by exotic (above) and native (below) insects per instantaneous sample of a plant to purple, white, or yellow; native and introduced flowers (+SEM).

2.4 Discussion

Results from my study suggest that the New Zealand flora may be inherently resistant to faunal invasion through native pollinator preferences for 'native' traits. This is because native pollinators were at a greater relative abundance in native-plant-dominated communities, native insects preferred native plants while exotic insects preferred exotic plants (when grouped), and flower colour can also be a driver of pollinator visitation in New Zealand. When insects were examined at the species level two of the three native bee species showed a significant visitation preference for native over exotic flowers. Both exotic bees preferred purple over white flowers, while the native syrphid fly, *Melangyna novaezelandiae,* visited fewer yellow flowers than while or purple flowers. None of the 7 most common insects showed a significant visitation preference for yellow over white flowers or exotic plants over natives. This suggests the native flora will be resistant to invasion because native pollinators will preferentially visit native (white) flowers and so exotic plants in these areas may suffer pollen limitation. Additionally, exotic pollinators are at lower relative abundance in native-plant-dominated areas and so their willingness to visit exotics will have less of an impact because they will not be providing a significant proportion of insect pollinator services.

The predictors of flying-insect visitation in New Zealand

Plant and insect provenance predict visitation

The relative abundance of native and exotic insects changed depending on the site of observations. This suggests that the wider plant community played a role in determining where insects foraged. Native invertebrate pollinators were relatively more common in the Port Hills, which is a site of secondary native forest, while exotic insect pollinators were more abundant at the two sites within Christchurch city. This supports findings in Australia which found differences in the distribution of bumblebees and honey bees depending on the location of floral substrate examined (Goulson *et al.* 2002). While Goulson *et al.* (2002) acknowledged that a few of these bees may be found throughout Tasmania they maintained that these exotic bees were in a far greater abundance in cultivated areas dominated by exotic plants. Hingston (2006) found that *Bombus terrestris* is well established in native vegetation in Tasmania. However, I found that exotic species were not at high relative abundances in native-plant dominated areas in urban Christchurch.

Hanley and Goulson (2003) found that introduced bees prefer introduced plant species after reviewing the current literature and added another season of data collected in New Zealand. Supporting this, my data show that native pollinators prefer native plants and exotic pollinators prefer exotic plants when grouped together. Clearly the provenance of the flower is not the literal predictor of visitation and some underlying combination of traits will be driving the correlation between plant and pollinator provenance. Groups of plants may demonstrate a common set of traits that have adapted locally. These traits likely correlate with their native or exotic status but pollinators will have been making choices based on preference for an underlying trait. One of these underlying traits may be petal colouration which is explored in more detail below (and in chapter 3 of this thesis). However, when insects were considered by species results were less consistent. Two of the native bees (*Leioproctus* and *Lasioglossum*) showed a significant preference for native species

over introduced species, while the remaining five insect species lacked a significant preference for either. Considering insects separately is important because this allowed for differences between days, between insect species, and the nested design of the observational data to be accounted for. A lack of preference for native or exotic flowers at the visitation level for most species makes sense because visitation is more specific and there is no such 'native' or 'exotic' trait.

Colour as a direct driver of visitation

An underlying mechanism of the preference of grouped native insect flower visitors for native plants and exotic pollinators for exotic plants is that of floral colour. Flower colours interact with plant-insect provenance in driving pollinator preferences. My broad tests showed exotic flower visitors preferentially visited coloured exotic flowers, while native insect pollinators preferentially visited white native flowers. Because native plants often have small simple white displays (Webb & Kelly 1993; Newstrom & Robertson 2005) this may also afford some protection from exotic pollinator establishment in less disturbed areas.

The preference of native invertebrates for native white flowers could be the result of frequency-dependent selection by pollinators (Smithson & Macnair 1997; Gumbert & Kunze 2001; Eckhart *et al.* 2006). This is particularly so if native flowers follow trends in colour and morphology as suggested by Lloyd (1985). Frequency-dependent selection by *Bombus terrestris* for common flowers over a rarer morph where both provided the same sucrose reward was shown in a study conducted in Exeter, UK (Smithson & Macnair 1996). Flower constancy (see Chapter 1, this thesis) may also play a role in the preference for familiar traits through a trade-off between foraging speed and accuracy (Chittka *et al.* 2003; Chittka *et al.* 2009). In this instance the common traits of the New Zealand flora may be the result of other factors such as pleiotropic traits (see Chapter 5, this thesis), and New Zealand's native fauna may then have developed an apparent preference for these groups of traits. This may match either Darwin's interference hypothesis: visiting the common morphs reduces handling times, or the search image hypothesis: specialising on 'runs' of one type allows them to process high volumes of information rapidly (Gegear & Laverty 2001). Therefore, while I have observed that native insects visit native white flowers more frequently, and exotic insects visit coloured introduced flowers, further exploration of the underlying mechanisms of these trends is needed before visitation can be extended to preference.

Insect species preferences were again different to general trends. When a preference for colour was detected both exotic bees, *Apis mellifera* and *Bombus* taxa, preferred purple flowers over white; while the syrphid fly, *Melangyna novaezelandiae*, preferred white flowers over yellow. None of the native bees showed a significant preference for colour when yellow or purple flowers were compared against white flowers. The preference of exotic bees for purple may have been the result of a greater ability to perceive these colours, as was believed to be the case with the Exeter study mentioned above which found *Bombus terrestris* showed a preference for blue over yellow flowers regardless of frequency (Smithson & Macnair 1996). Their result is consistent with *Bombus'* preference for purple over white in my study. A lack of colour preference of native New Zealand bee pollinators is consistent with earlier claims that New Zealand's pollinating invertebrates lack colour selectivity. However, *Melangyna novaezelandiae's* (syrphid fly) preference for white flowers contrasts with Campbell *et al.*'s (2010) study which found the native syrphid flies (*Allograpta* and

Platycheirus) to prefer yellow over white flowers when preferences were detected. Therefore the colour preferences of New Zealand's flower visiting invertebrates warrant further investigation, which will be done in the following chapter of this thesis (chapter 3).

Limitations of this study

There are a number of limitations of this chapter that need to be considered. The data collected were observational and so data are unbalanced (both within species and between native and exotic plants), across a range of conditions, sites, dates, and plant traits. There was a great disparity between plant species, with 25 introduced, and 14 native plant species included. Along with this, an uneven number of plant replicates per plant species was observed, as some plants were available in abundance, while 14 species only had a maximum of one flowering plant from which observations were taken at a site. This resulted in the inclusion of 1623 introduced plants and 445 native plants. Conditions also varied as observations took place across a 4 month period at 3 different sites, and while data on temperature, wind, shade levels, and cloud cover, were collected, it is possible that this was still not sufficiently accounted for. Finally, plants with differently sized flowers or displays were not removed. Therefore it is difficult to be certain if size or shape or some other morphological factor was misleading results. Some insect visitors may have been recorded on exotic plants more frequently because these tend to have more flowers, therefore leading to skewed results (Hingston 2005). A subset of large bushes was observed. However there still may be bias simply because bushes with large displays were more enticing to pollinators. Because this was an observational rather than experimental chapter such variances were difficult to control for, beyond that which the statistical model has the power to do. However, observational work is important because it gives a picture of occurrences under real-life conditions which can then guide the design of manipulative experiments. The experimental analysis in the following chapter serves to account for the discrepancies noted in this one.

It must be noted that visits do not necessarily lead to pollination. Some insect species may be cheating (Kelly *et al.* 2006), not depositing pollen on receptive stigmas (such as between species or within plants), or using the flower to rest. When pollination does occur this is not necessarily at a high quality as some pollinating species may frequent flowers but rarely deposit pollen (Bischoff *et al.* 2012). A suggestion for a similar study would be to track pollen removal and deposition, and quantify the differences in pollination quality as was done by Bischoff *et al.* (2012). While other mutualists such as the avifauna of New Zealand also play a role in pollination, few were observed during this data collection, despite occasionally sitting some distance away for long periods to see whether other visiting classes may come. Webber *et al*. (2012) also found that bird visits were comparatively rare in the Christchurch Botanical Gardens.

The resistance of the New Zealand flora to the establishment of exotic species

Brockerhoff *et al.* (2010) suggested that the New Zealand flora may be resistant to invading fauna because of its phytogenic distinctiveness and host specificity of invading species; the biotic resistance hypothesis. This states that undisturbed habitats tend to be less likely to suffer invasions than their equivalent invaded habitat (Brockerhoff *et al.* 2010). Most of the exotic bee pollinators established in New Zealand were introduced here deliberately for agricultural and crop pollination. One of these species, *Bombus terrestris,* has been particularly successful at increasing its range and

forming wild colonies (Donovan 2007; Brockerhoff *et al.* 2010). The findings of this chapter demonstrate that native pollinators will more frequently visit native white flowers while exotic pollinators are more commonly seen visiting coloured exotic floral displays. The prevalence of exotic pollinators in urban areas, and their strong preferences (when grouped) for coloured exotic flowers suggests that these plant invaders will not suffer pollen limitation when exotic pollinators are present. However, because of the preferences of exotic pollinators, introduced-plant establishment will likely only occur in areas of high disturbance. This is because New Zealand's exotic pollinators are at greater relative abundances in urbanised areas (data this chapter). Were these pollinators able to become established within dense native flora then they would likely facilitate the invasion of weeds (Hanley & Goulson 2003). However, because native pollinators continue to prefer native plants, exotic weeds which do not imitate common traits of the native fauna might suffer pollen limitation in native-plant-dominated areas. Therefore, at least for the time being, the low relative abundance of exotic pollinators in native areas may mean that the native flora will continue to resist the establishment of coloured exotic plants.

Less encouraging is the preference of native pollinators for white flowers, even those with an exotic provenance. Conservation efforts may wish to focus on the removal of white exotic plants from native foliage. Conversely, this may mean that native insect pollinators will supplement agricultural pollination of plants whose flowers have native-like (small, simple, white (Lloyd 1985)) traits.

Chapter 3

The effect of flower colour on the visitation of New Zealand's pollinating insects

3.1 Introduction

Petal colour can mediate the interaction between invertebrate pollinators and their plants. Flower visiting insects may show strong preferences for flowers of a particular colour and visit these more frequently than other colour morphs. Pollinating animals are believed to have an influence on floral traits through their preferences and visual capabilities as these species are mobile and have the cognitive capacity to choose. In New Zealand, insect visitors play an important role in pollination, with a combination of exotic and native invertebrates contributing to the pollination services provided. The plants of New Zealand have some characteristics distinct from the flora of other continents such as small simple flowers (Webb & Kelly 1993); often white, with dish or bowl shapes (Lloyd 1985; Newstrom & Robertson 2005) and small fruits (Lord *et al.* 2002). A traditional belief in the New Zealand literature is that New Zealand's common floral traits have developed as a result of undiscerning pollinator visitors (Webb & Kelly 1993). This view has been recently challenged by the work of Campbell *et al.* (2010, 2012) who demonstrated sensitivity to flower colour among some of New Zealand's native alpine pollinators. This colour sensitivity was sometimes for colours other than white. Therefore the colour preferences of New Zealand's flower visiting insects need further investigation.

In chapter 2 I showed some effects of flower colour and plant provenance on the visitation of New Zealand's flower visiting invertebrates. When pollinators were grouped according to their provenance native pollinators showed a preference for native plants coloured white while exotic pollinators preferred exotic non-white flowers. However these results were observational and so some confounding factors were unable to be separated from results. Hence in this chapter I set out to experimentally alter plant colour and plant provenance in an urban setting. Addressing the effect of flower colour on insect flower visitation in an urban setting is interesting because the stronger blend of native and exotic insect species is becoming more common. This is important because the increasing abundance of exotic insects will mean they will have a greater influence upon pollination in New Zealand in the future. It is expected that trends in this chapter will support grouped observational data from the previous chapter; with exotic flower visiting insects preferring not-white exotic flowers and native flower visiting insects preferring white native flowers.

Factors driving pollinator visitation

The ability of pollinating invertebrates to learn

To understand the decisions foragers make it would help to summarise a few brief points about the ability of flower visiting insects to learn. While species are different, most learn through a number of similar mechanisms; habituation, sensitization, and associative learning (Weiss 2001). A number of studies exist that have demonstrated associative learning in fly species (Weiss 2001). Sheep blowflies can associate colours with rewards, *Drosophila* species are capable of responding to

olfactory conditioning, and hoverflies can use landmarks as visual cues (Weiss 2001). Lepidopterans can associate odours, tastes, shapes, and colours; and those trained on colour associations are capable of quickly reversing the association, demonstrating true learning (Weiss 2001). As with hovering flies, some Lepidopterans (Weiss 2001) and Hymenoptera (Wehner *et al.* 1996) can also use landmarks as visual cues. Sometimes innate preferences are stronger than learned preferences. This is seen in some syrphid flies which can be trained in colour associations but this association is not as strong as their innate preference for yellow flowers (Weiss 2001). Similarly, when a Swedish study tested the visual preferences of the hawkmoth, *Macroglossum stellatarum*, for different patterns the authors found this species was sometimes unable to develop a conditioned preference for one pattern over an innate preference for another, depending on the patterns (Kelber 2002). Therefore insect pollinators will make decisions differently based on their capacity for learning.

Plants have evolved to manipulate pollinator learning. One way to do this is to force insects to learn how to access unusual floral shapes. For some generalist species such as *Apis mellifera* and *Bombus* species, handling times decrease with experience; the more frequently they visit one flower morph the better they become at obtaining the rewards. Therefore flowers with complex morphologies may gain recurrent visitors as it is more advantageous for the insect species who visit them to specialise on a few morphs rather than relearning reward access many times (see flower constancy in Chapter 1) (Menzel 2001; Weiss 2001). Another advantage of pollinator learning (to plants) is that of pleiotropic traits (where a single gene controls a number of phenotypic traits) such as reward signals. Some angiosperms change the colour of their flowers to inform visitors about the level of their current rewards. In this instance when resources are depleted the flower changes colour, signalling to the pollinator that they are no longer worth visiting (Weiss 2001). Pollinators have been shown to preferentially visit the pre-change colour-morph of these floral species (Weiss 2001). This results in more efficient pollination services as foragers do not waste time on plants without pollen to transfer. Therefore the ability of insect pollinating species to learn may occasionally be plastic with the state of the plants they visit. The ability to learn likely flourishes under changing environments (Weiss 2001), and because it allows a pollinator to better adapt in changing environments it may be assumed that this will continue to be selected for in the future.

The visual capabilities of invertebrate pollinators

The visual capabilities of floral visitors such as Hymenoptera can play a major role in determining foraging habits as they allow an animal to discriminate. This discrimination can affect flower constancy (Giurfa & Lehrer 2001), pollination success, and floral evolution. Colours expressed are often those which are most easily distinguished by the pollinators who visit them. These colours appear to have been constrained by pre-existing gene capabilities rather than coevolved alongside pollinator vision (Dyer *et al.* 2012). Flower colour is significantly correlated with the visual capabilities of Hymenopteran species. One of the best studied hymenoptera is the honeybee, *Apis mellifera*, which has trichromatic colour vision (Giurfa & Lehrer 2001). This means that they have three different types of spectral sensitivity which peak at three wavelengths (Giurfa & Lehrer 2001). An example of flowers co-varying with the visual capabilities of their pollinators has been seen in plants from Maranoa Gardens, Melbourne, Australia. The colours of the 111 native plants collected were shown to co-vary similarly to the ability of Hymenoptera to discern different colours (Dyer *et*

al. 2012). Therefore, the vision of Hymenoptera plays an important role in both flower choice, and plant adaptations.

Other pollinating invertebrates also have a range of visual capabilities. While Hymenoptera have trichromatic vision, butterflies can be trichromatic, tetrachromatic, or pentrachromatic (Dyer *et al.* 2012), leading to some of the broadest spectral range of any known animal species across UV to red colourations (300-700 nm) (Weiss 2001). Butterflies are believed to use visual cues to associate flowers with rewards (Weiss 2001); as are the thrips, *Frankliniella schultzei*, which are able to detect colours mainly in the red reflectance range (600-700 nm) (Yaku *et al.* 2007). The hawkmoth, *Macroglossum stellatarum,* has been shown to possess the visual capacity to detect patterns; a visual cue that is preferred over uniform patterns (Kelber 2002). The ability of flies to discern different colours is often poor (Dyer *et al.* 2012), although it has been shown that some genera of fly are capable of distinguishing colours (Weiss 2001). The visual capabilities of invertebrate pollinators vary across species, genera, and families; so generalisations are difficult. Importantly, the visual capabilities of pollinating species may drive the pollinator preferences observed worldwide.

The colour preferences of invertebrate pollinators

Floral colouration and pollinator colour preferences are often tightly linked, although other factors may at times prevent this (Chittka *et al.* 2001). The colour preferences of invertebrate pollinating fauna have been studied around the world; and UV, green, and blue receptors are believed ancestral to vision in all of Insecta (Weiss 2001). Syrphid, calliphorid, tephritids and anthomyiid flies are all thought to prefer yellow coloured flowers over other colourations, while bee-flies (bombyliids) are more versatile and are commonly seen upon the pink to purple and blue range (Weiss 2001). Lepidopterans vary in their visual capabilities with some species only able to perceive a few colours while others may be able to perceive several (Weiss 2001). While their colour preferences do differ across species, a generalisation can be made that foraging Lepidoptera species will often preferentially visit blue or yellow-red flower morphs (Weiss 2001).

The preferences of invertebrate pollinating fauna can influence the direction of petal colour adaptations. The colour preferences of the butterfly, *Aeropetes tulbaghia*¸ have been shown to lead to localised colouration of the non-rewarding mimetic orchid, *Disa ferruginea*, in South Africa (Newman *et al.* 2012). Here butterflies preferred orange colour morphs in areas where rewarding plants were also orange and red morphs where rewarding flowers were red, resulting in mimetic orchid colourations often matching these preferences. This demonstrates local colour adaptation driven by pollinator preference (Newman *et al.* 2012). An American study which looked at twolobe larkspur, *Delphinium nelsonii,* found that bumblebee and hummingbird pollinators tended to discriminate against white forms of the usually blue flowered plant, and this white colour was then shown to correlate with longer handling times at each flower, thus reducing foraging efficiency (Waser & Price 1983). Colour preferences of the invertebrate pollinating fauna are diverse and inherently linked to visual capabilities and correlations between phenotypes and expected rewards.

The attributes of New Zealand's flora and fauna

As outlined in Chapter 2, the native New Zealand flora and fauna have a number of common attributes. Notably, the flowers are commonly simple (Webb & Kelly 1993), and are often white

(Lloyd 1985). The reasons behind this are not obvious however and previously the small, simple, white, dish-shaped features of New Zealand's flowers have been hypothesized to result from the predominance of fly pollination (Newstrom & Robertson 2005). New Zealand has a combination of native and exotic insect pollinating species. The small native bees are all solitary and often nest in the long tunnels, or within twigs and branches found on the ground (Crowe 2002; Donovan 2007). These native bees have been said to lack colour selectivity (Wardle 1978; Lloyd 1985). New Zealand's exotic insect fauna also plays a role in pollination (Kelly *et al.* 2006). There have been numerous studies of exotic bee foraging habits such as the ability of *Bombus* species to make foraging decisions based on the quality of available pollen (Bronstein *et al.* 2006), and the innate preferences of *Bombus* species for blue or purple coloured flowers (Gumbert 2000). However, innate preferences for certain colours have been shown to quickly diminish through learning (Gumbert 2000). For a more detailed examination of the native and exotic fauna see Chapter 2. While some literature on New Zealand's insect pollinating taxa has been published there is currently little published on the colour preferences of New Zealand's native fauna.

The colour preferences of New Zealand's pollinating insects

In New Zealand insects are important pollinators, and recent research suggests these pollinators have colour preferences. The colour preferences of two genera of New Zealand syrphid flies, *Allograpta* and *Platycheirus,* and two native solitary bees, *Hylaeus* and *Lasioglossum* species were examined. When a preference for colour was detected both flies chose yellow over white flowers, while *Hylaeus* species preferred the white colouration of *Ourisia glandulosa* only (Campbell *et al.* 2010). Therefore plant species influenced colour preference. The idea of multiple traits driving pollinator visitation supports Gegear and Laverty's (2001) 'trait variability hypothesis' (see flower constancy, Chapter 1) as visitation depended on a number of different aspects of the flowers they could visit. Therefore pollinators may be exhibiting flower constancy to some degree, and this may have an impact on the expressed preferences of flower visitors included in my study.

Campbell *et al.*'s (2010) work on native pollinator colour preferences was expanded to determine whether the colour of flowers had an impact on both visitation and pollen export. It was found that bees changed their floral preference based on the size of flowers when visiting *Wahlenbergia albomarginata* (Campbell *et al.* 2012). It was also found that a preference for blue painted flowers existed at a larger spatial scale but was not present at a smaller scale. Increased visitation lead to an increase in pollen export (Campbell *et al.* 2012). However their study was unable to demonstrate any preference for colours in the natural range shown by the pollinating solitary bees of *Wahlenbergia albomarginata*, an alpine herb native to New Zealand (Campbell *et al.* 2012). This emphasises how difficult it is to isolate preferences as visitors experience an array of stimuli at once and may make foraging decisions based on any number of these. It also shows how difficult it is to create experiments that are relevant in natural settings, therefore suggesting both experimental and observational work is needed. The investigation into the colour preferences of pollinating species is on-going worldwide and much is still to be learnt about the colour preferences of New Zealand's pollinating fauna.

In Chapter 2 I gained insights into the preferences of New Zealand's insect pollinating fauna through an observational study. In this chapter an experimental study was designed to test these findings.

By conducting a controlled experiment the natural variation unable to be accounted for in the previous chapter can be managed, and empirical data on New Zealand's insect pollinator preferences is gained. Quantifying the impact of invasive insects is difficult because of the large number of factors involved, and so often small experiments yield clearer results than large observational studies (Thomson 2006). An urban environment has been chosen to extend Campbell *et al.*'s (2010; 2012) findings across different communities, and to determine whether native insect pollinators behave differently in the presence of abundant exotic insects. This is particularly relevant because urban environments are ever expanding and it is important to be able to predict the impact this will have on the current flora and fauna.

The Christchurch Botanical Gardens were chosen as the site for a number of reasons. Firstly, these gardens have sections dedicated to both native and exotic plant species therefore guaranteeing abundant plant resources; secondly they are in an urban environment, yet still sufficiently plant-dominated to ensure abundant insect pollinators across both exotic and native origins; and thirdly, because I have already assessed the impact of location on the flower-visiting behaviour of pollinating invertebrates at the Botanical Gardens in Chapter 2.

Chapter objectives

Findings from the previous chapter indicate a trend in pollinator preferences. When flower visitors were grouped according to their native or exotic origin, native visitors preferred white native flowers while exotic insects preferred coloured exotic flowers. This was then narrowed to individual flower visiting taxa and results were much more complex, with particular flower visiting species showing some preferences for certain colours. This could have been the result of a number of underlying characteristics and the intention of this chapter is to test the simplest hypothesis: native pollinators prefer white flowers while exotic pollinators prefer non-white flowers. This chapter is restricted to two colours only, yellow and white, to try and isolate the underlying mechanisms driving pollinator preferences in New Zealand. Purple flowers are not used due to time constraints and plant availability.

This study has four questions:

1. Do native pollinators prefer white flowers and exotic pollinators prefer yellow flowers?
2. Do native pollinators prefer native flowers and exotic pollinators prefer exotic flowers or lack a preference?
3. Is the strength and direction of the colour preferences of New Zealand's insect visitors dependent to some degree upon the provenance of the flower visited?
4. Do pollinators prefer a familiar set of traits?

These questions are tested by counting the invertebrate flower visitors to six different plant species in controlled arrays over one hour periods. Experiments used an array of 8 native and 8 exotic flowers of different colour combinations arranged randomly in a 30 cm square grid.

3.2 Methods

Study site and species

I used the Christchurch Botanical Gardens (43°53'S, 172°62'E) as my study site. The Christchurch Botanical Gardens are located within Christchurch, a few kilometres from the city centre. This site has a mixture of garden types (ranging from native bush to exotic herbaceous borders), and was used for all data collected in this chapter. A single location was chosen to ensure that the variation in relative abundance of different pollinators found at different sites (as demonstrated in Chapter 3 of this thesis) did not lead to a misleading result. Data were collected in two areas within the Botanical Gardens, one near the New Zealand Gardens (native flora) and one near the Herbaceous Border (exotic flower species) in an attempt to sample the range of varying relative pollinator abundances.

For unpainted trials exotic plant species used were *Artemisia lactiflora* (Asteraceae) which is a herbaceous perennial that produces off-white flowers, *Helenium flexuosum* (Asteraceae) which is a compound Asteraceae inflorescence with deep yellow ray flowers and much darker disk flowers, and *Geranium renardii* (Geraniaceae) which is a white colour with a few light pink markings; similar in size to *Helenium flexuosum* (Fig. 3.1). Native plant species were *Hebe salicifolia* (Plantaginaceae) which produces a cluster of white florets, and two compound Asteraceae inflorescences: *Pachystegia insignis* (Asteraceae); a large flower with white rays, and an *Olearia* (Asteraceae) cultivar with smaller yellow ray flowers (Fig. 3.1). Ideally a cultivar would not have been used in this experiment, however, a lack of available native yellow flowers made this impossible. Finally, for painted trials the yellow exotic *Helenium flexuosum* and native white *Pachystegia insignis* were used.

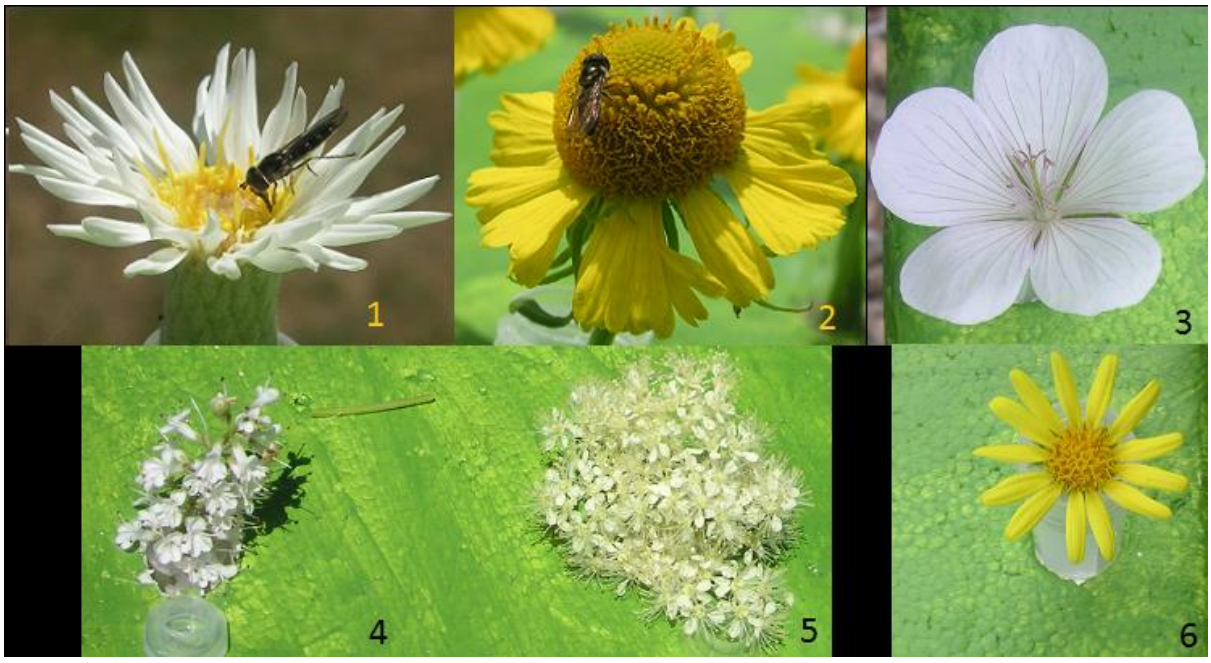

Figure 3.1: Six flowers used in array experiments. In order from left: *Pachystegia insignis, Helenium flexuosum, Geranium renardii, Hebe salicifolia, Artemisia lactiflora, Olearia* cultivar. Relative scale: 1 & 2: a *Melangyna novaezelandiae* visitor, 3 & 6 same photo, 4 & 5, same photo.

The exotic plant species used were all collected from the Herbaceous Border of the Christchurch Botanical Gardens, hereby referred to as the exotic section. Native plants were all sourced from the

New Zealand Gardens section of the Christchurch Botanical Gardens hereby referred to as the native section. I had hoped to use the same species pairs throughout the experiment but changes in what flowers were available as the season went on made that impossible.

One-hour insect counts

Insects were counted over an hour-long period for each array. Flowers were numbered 1-16 and all flying invertebrates rested upon flowers were included. For each visit I also noted visited-flower location (in case there were edge effects) and a visit was considered finished when an insect left the array box perimeter. Some individuals may have been counted more than once as they left and returned to the array, but there was no way to track individual insects. A total of 4 different plant-colour combinations were used for unpainted trials (16 arrays in total) and one painted array was repeated 7 times. Each array was abandoned if there were no visits within 30 minutes.

General methodology

Methods were based on those used by Campbell *et al.* (2010, 2012). Floral arrays were set up of 16 flowers in a 4x4 arrangement, consisting of 8 native and 8 exotic flowers. Flowers were coloured either yellow or white (or half of each) depending on the array run and each array was repeated at least four times.

Flowers were placed in plastic eppendorf tubes which sat in a polystyrene box ~120 cm high (held on four fence standards). Because it was not possible to set up the arrays near local patches of both source plants, array locations were alternated equally between the exotic and native sections. This was to ensure that the flowers found were equally novel to both native and exotic flower visitors. Therefore the community context always included one of the species being used in the arrays and on a larger spatial scale a range of native and exotic flowers.

All arrays were run between 11am and 4.30pm on sunny days above 16 degrees but below 28 degrees, with wind no greater than 6 ms^{-1}. Arrays were placed in direct sunshine within 5 m of a source plant.

Natural flowers

Unpainted arrays were run from 14 December 2012 until 13 January 2013. For these arrays the first species pair consisted of *Hebe salicifolia* as the flower source native to New Zealand, and *Artemisia lactiflora* as the exotic flower species (Fig. 3.1). The second species pair used a yellow exotic, *Helenium flexuosum,* and a white native, *Pachystegia insignis*. This was followed by a yellow exotic and a yellow native, *Helenium flexuosum* and an *Olearia* cultivar; and finally by a white exotic and a yellow native, *Geranium renardii* and an *Olearia* cultivar**.**

Table 3.1: Plant species pairs, colours, and replicates for painted and unpainted array experiments following preliminary trials. These experiments were used for analysis.

Manipulation	Species 1 (exotic)	Species 2 (native)	Colours (1, 2)	Replicates
Not painted	*Artemisia lactiflora*	*Hebe salicifolia*	White, white	4
Not painted	*Helenium flexuosum*	*Pachystegia insignis*	Yellow, white	4
Not painted	*Helenium flexuosum*	*Olearia* cultivar	Yellow, yellow	4
Not painted	*Geranium renardii*	*Olearia* cultivar	White, yellow	4
Painted	*Helenium flexuosum*	*Pachystegia insignis*	Yellow, yellow	7

Painted flowers

Painted arrays were run from 14 January 2013 until 22 January 2013 using *Helenium flexuosum* and *Pachystegia insignis* (Table 3.1). Flower petals were painted on the top side only with a single coat to try and maximise both colour cover and the durability of the flowers so that they would not wilt before the hour long observation period was completed.

Acrylic paint has been used in similar experiments with no detectable changes in New Zealand native insect pollinator visitation as result of possible changes in fragrance or blocking UV reflectance (Campbell *et al.* 2010, 2012). Therefore white and yellow acrylic paint was chosen for the painted trials in this study.

Preliminary trials before data were collected

My original intention was to have three pairs of flowers of similar morphology which would be painted in different combinations. However, difficulties in both flower availability and poor visitation meant that most of the season was spent attempting to attract bees rather than applying treatments, and these preliminary trials are covered in more detail below. In sum:

In November 2012, *Pratia angulata* and *Geranium renardii* were selected as the first plant pair. These species were chosen because they were both predominantly white, had a large number of flowers available, and allowed the use of a native and exotic flower species in the same array. This array plant-pair was run with neither of the flowers painted (Fig. 3.2), both species painted white, one painted white and one painted yellow (and vice versa), and both painted yellow; with two *Lasioglossum* and one *Parentia* (native fly) visit in total (all to *Geranium renardii*) across all five array experiments run (Table 3.2). Following these trials a number of different attributes of the arrays were manipulated to try and isolate the feature which was discouraging visitation. Arrays were run which altered height, array location, plant species, materials, paint brand (as well as discontinuing paint use altogether), and using gloves to reduce any unknown impact of handling.

Figure 3.2*: Geranium renardii* and *Pratia angulata* in a preliminary trial where flowers were not painted.

In early December eight arrays were run using *Achillea millefolium* (exotic plant) and an *Olearia* cultivar (native plant) (Table 3.2). Flowers were initially painted yellow with new acrylic paint from THE WAREHOUSE (Brand: FAS fine art supplies. Colours: white and lemon yellow). This array received one visit by a *Lasioglossum* bee before paint was applied and none hence. A second array was run that afternoon without paint, with again only one *Lasioglossum* visitor. The following day an array was run to test whether the polystyrene box being used had any impact on visitation. In these two arrays test tubes were trialled in a thick short hedge adjacent to the exotic source plant and later test tubes were stuck in a lawn adjacent to the native source plant. Neither of these trials received any visitation. This was repeated with more flowers per test tube to see if display size was the issue, again no visits. The next array trial involved using larger umbels of *Achillea millefolium* in eppendorf tubes to ensure the glare of the plastic test tubes was not deterring visitors. This was repeated twice, both times with only a few *Lasioglossum* visits (in total: 3 to *Achillea millefolium,* 1 to *Olearia*).

Following this, flowers were painted directly without removing them from their source plant to see whether equipment, handling or another issue such as insect seasonality was impacting visitation, however the temperature on this day surpassed 30 degrees and bee activity was noticeably reduced. No visits were observed (Table 3.2). Location was then examined by performing a number of arrays on a private farm in Avoca Valley (43°34’S, 172°41’E) which is approximately 7 km east of the Botanical Gardens. Here the area used was full of long grasses and wild flowers, and was abundant in native and exotic bees. Plant species used for arrays in this location were elderberry (*Sambucus*) and creeping *Muehlenbeckia*. Arrays were placed in the paddock within a few metres of source plants, and surrounded by hundreds of bees. Amazingly, despite an abundance of bees to the point where bees landing on my clothes was commonplace, arrays received no visits (Table 3.2).

Table 3.2: Manipulations used on plant pairs during preliminary trials and the visitation by flower visiting insects. Insects are recorded visiting plant 1 or plant 2. These data are not included in analysis.

Manipulation	Species 1 (exotic)	Species 2 (native)	Colours (1, 2)	Repeats	Visits
None	*Geranium renardii*	*Pratia angulata*	White, white	1	*Lasioglossum* (1 to 1), *Parentia* (1 to 1)
Painted	*Geranium renardii*	*Pratia angulata*	White, yellow	1	None
Painted	*Geranium renardii*	*Pratia angulata*	Yellow, white	1	None
Painted	*Geranium renardii*	*Pratia angulata*	White, white	1	None
Painted	*Geranium renardii*	*Pratia angulata*	Yellow, yellow	1	*Lasioglossum* (1 to 1)
None	*Achillea millefolium*	*Olearia* cultivar	White, yellow	1	*Lasioglossum* (1 to 1)
Painted	*Achillea millefolium*	*Olearia* cultivar	Yellow, yellow	1	None
None	*Achillea millefolium*	*Olearia* cultivar	White, yellow	1	*Lasioglossum* (1 to 2)
Box: Hedge	*Achillea millefolium*	*Olearia* cultivar	White, yellow	1	None
Box: Lawn	*Achillea millefolium*	*Olearia* cultivar	White, yellow	1	None
Display size	*Achillea millefolium*	*Olearia* cultivar	White, yellow	1	None
Eppendorf tubes	*Achillea millefolium*	*Olearia* cultivar	White, yellow	2	*Lasioglossum* (5 to 1, 1 to 2)
Flowers on plant	none	*Olearia* cultivar	White & yellow	1	None
Avoca Valley	*Sambucus*	*Muehlenbeckia*	White, whitish	4	None
Height/paint	*Helenium flexuosum*	*Olearia* cultivar	Half of each painted white, unpainted flowers: yellow, yellow	1	*Bombus* (3 to 1, unpainted), *Parentia* (2 to 1, unpainted), *Lasioglossum* (19 to 1, 7 to 2, painted), *Hylaeus* (9 to 1, painted), *Lasioglossum* (24 to 1, 8 to 2, unpainted), *Hylaeus* (6 to 1, 3 to 2 unpainted)
Display size	*Helenium flexuosum*	*Olearia* cultivar	Yellow, yellow		*Lasioglossum* (8 to 1, 3 to 2), *Hylaeus* (6 to 1, 4 to 2), *Melangyna novaezelandiae* (1 to 1, 5 to 2), *Apis mellifera* (3 to 1), *Bombus* (1 to 1), *Eristalis tenax* (1 to 2)

In mid-December 2012 arrays were relocated back to the Botanic Gardens. Fence standards were attached to the polystyrene box of arrays to a height of ~120 cm and flower species used were

Helenium flexuosum and an *Olearia* cultivar. Half of the flowers were painted white (four *Helenium flexuosum* and four *Olearia* flowers), while the remaining flowers were left unpainted. These arrays received 81 visits in total; primarily Lasioglossum and Hylaeus with no obvious preference between painted and unpainted flowers (in total: 35 visits to painted flowers and 46 to unpainted flowers) (Table 3.2).

The following array on this day used *Helenium flexuosum* and an *Olearia* cultivar in bunches to try and match the display size of the slightly larger exotic at the same height as the previous trial. No flowers were painted and visits were a reasonably well represented mix of native (27) and exotic species (5) (Table 3.2). Because of the lateness of the season I decided to persevere with unpainted flowers to ensure sufficient replication of exotics as well as natives for testing, and experiments only moved to include painted arrays in mid-January. This meant that painted arrays were limited to only one plant pair (*Helenium flexuosum* and *Pachystegia insignis*) painted yellow, therefore replication was reduced. None of the preliminary array trials were included in analysis because these data were collected earlier in the season under different conditions.

Statistical analysis

Because of the low visitation for painted arrays most of the analysis concentrates on unpainted arrays. While visitors were different, in both array types visitation consisted of nine flower visiting insect taxa across six families. I pooled data into native and exotic insect groups to determine whether native flower visitors preferred white flowers and exotic visitors preferred yellow, whether native flower visitors preferred native flowers and exotic pollinators preferred exotic, and whether the strength and direction of these preferences depended to some degree upon each other. This was done as a result of the low visitation of some insect taxa.

The array type (array: whether flowers were all white, all yellow, yellow native and white exotic, or white native and yellow exotic) and location within the gardens (arraylocation: near the native or exotic section) were included in the model as this allowed the model to account for the plant species used, the particular type of array (whether all yellow, all white, or a combination of white and yellow flowers), and whether native or exotic insects were at different relative abundances. Initially the best fit model was determined by finding that with the lowest Akaike Information Criterion (AIC) value by comparing models with and without different random effects using likelihood ratio tests in R (i.e. the 'anova (model1,model2)' command). Random effects were also plotted to determine whether there was a significant difference between the different arrays used. Analyses were carried out using generalised linear models (glmer) in R statistical package (version 2.15.0 R Foundation for Statistical Computing 2012).

The final model was developed that had the lowest AIC Value:

```
Model1 <- glmer (count~ plantprovenance * flowercolour * insectprovenance+
(1|array) + (1|arraylocation) + (1|flowerspecies), family = poisson, data =
dataset)
```

Fixed effects included in analysis are the provenance of the plant (plantprovenance: native or exotic to New Zealand), the provenance of the insects (insectprovenance: native or exotic), and flower colour (flowercolour: white or yellow). The interaction between the fixed effects was also tested. Because my data were count data a Poisson distribution was used.

Whether flower visiting insects prefer a familiar set of traits was unable to be tested for because of the low visitation to painted flowers, however the mean number of visits to white or yellow flowers which were painted yellow have been graphed.

3.3 Results

Natural flowers

Array observations yielded 669 insect visits to unpainted arrays. Flower visitors included a range of both native and exotic bee and fly species across Colletidae, Syrphidae, Simuliidae, Dolichopodidae, Apidae and Halictidae families (Table 3.3). Bees seen most frequently were native *Lasioglossum* (379 visits) and *Hylaeus* species (117 visits).

Table 3.3: Proportion of insect visitors to unpainted arrays included in analysis. Exotic insects are indicated with a *

Array (exotic, native)	Insect taxa	Exotic plant	Native plant
Artemisia lactiflora, Hebe salicifolia	*Apis mellifera* *	3	1
	Lasioglossum	30	22
	Hylaeus	0	1
	Leioproctus	0	4
	Austrosimulium	2	0
Helenium flexuosum, Olearia species	*Apis mellifera* *	16	1
	Austrosimulium	1	0
	Bombus *	4	0
	Hylaeus	8	4
	Lasioglossum	63	22
	Melangyna novaezelandiae	15	5
Geranium renardii, Olearia species	*Hylaeus*	0	7
	Leioproctus	0	1
	Melangyna novaezelandiae	0	1
Helenium flexuosum, Pachystegia insignis	*Apis mellifera* *	4	1
	Austrosimulium	1	2
	Bombus *	3	21
	Hylaeus	43	54
	Lasioglossum	142	100
	Leioproctus	27	21
	Melangyna novaezelandiae	5	33
	Parentia	1	0
Grand total		**368**	**301**

Results of the AIC comparison of different models showed that the model with random terms for the array, the array location, and flower species had the lowest AIC value, and this was significant using ANOVA. Plotted residuals supported this as Model1, which included the interaction term, was again shown to best fit the data as this plot had the least outliers and lowest non-normality of the residuals. The plot of random effects was found to also be significant. This is important to note as the random effects were significant (i.e. non-zero) which may be misleading the results of the fixed effects (plant provenance, insect provenance and flower colour).

Whether the colour pigmentation or the provenance of a flower played a significant role in the number of insect visitors was tested using a generalised linear model. Neither flower colour, flower provenance, insect provenance, nor the interaction of these, significantly predicted the visitation of the insect flower visitors (Table 3.4). This is surprising considering the results of the previous chapter and is likely the result of low visitation. This may be a cautionary tale on including only two colours in experimental arrays.

Table 3.4: Parameter estimates, standard error, z statistics, and *P*-values of the minimum adequate poisson GLMM (Model1) for all insect species, testing the fixed effects: flower provenance, flower colour, and insect provenance, and their interactions; while including the random effects of array, array location, and flower species in the model.

Fixed effects	**Estimate**	**Std. error**	**z value**	**Pr (>|z|)**
Intercept	-4.60	1.55	-2.96	0.003
Flower type (native)	1.87	1.38	1.36	0.18
Flower colour (yellow)	2.74	1.68	1.63	0.10
Insect type (native)	2.64	0.60	4.41	<0.001
Native flower: Yellow flower	-3.79	2.26	-1.68	0.09
Native flower: Native insect	-0.47	0.63	0.46	0.46
Yellow flower: Native insect	-0.38	0.63	0.54	0.54
Native flower: Yellow flower: Native insect	1.21	0.98	1.23	0.22

The lack of significant preference for white or yellow flowers in my experimental arrays is also demonstrated below (Fig. 3.3). While I received far more native flower visitors than exotic visitors (i.e. 54 of 669 visits were by exotic species) neither native nor exotic invertebrates show an obvious preference for white or yellow flowers. Similarly there was no clear difference in visitation for native or exotic plant species, depending on the flower visitors own native or exotic status (Fig. 3.4).

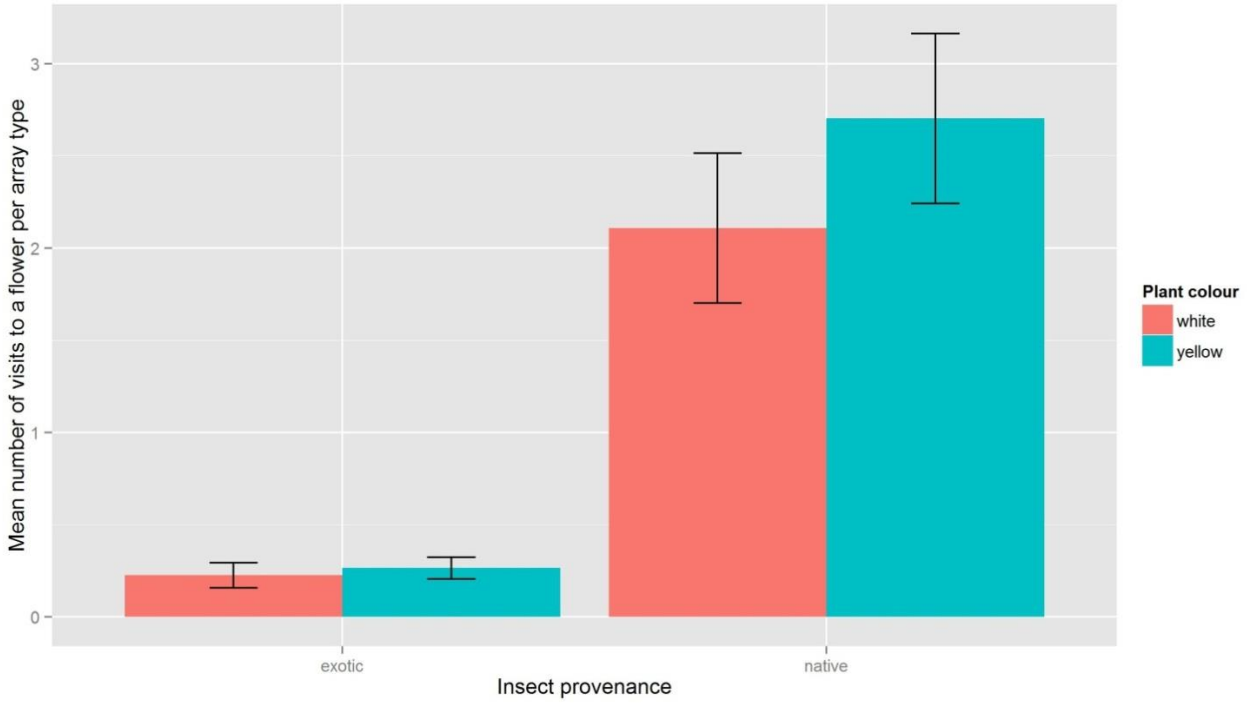

Figure 3.3: Mean number of visits to a yellow or white flower per array type (+SEM) by native and exotic insects at the Christchurch Botanical Gardens. Array types were all yellow, all white, white and yellow, and yellow and white.

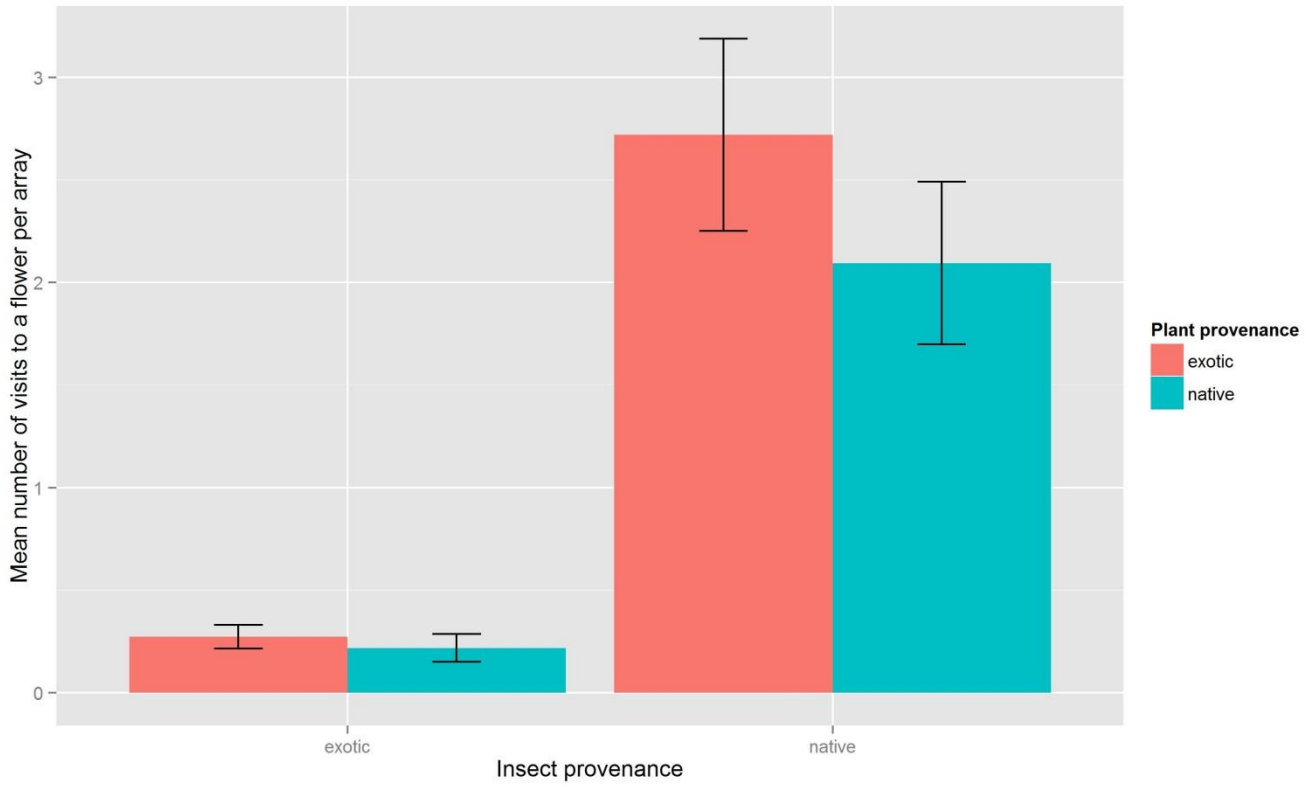

Figure 3.4: Mean number of visits to a native or exotic flower per array type (+SEM) by native and exotic insects at the Christchurch Botanical Gardens. Array types were all yellow, all white, white and yellow, and yellow and white.

Painted arrays

While I unable to be test whether pollinators prefer a familiar set of traits because of low visitation to painted arrays (79 insects) this figure does suggest that flower visitors may prefer familiar traits as yellow flowers that were painted yellow received greater comparative visitation than white flowers painted yellow for both native and exotic insects (Fig. 3.5), perhaps because this is closer to a familiar morph.

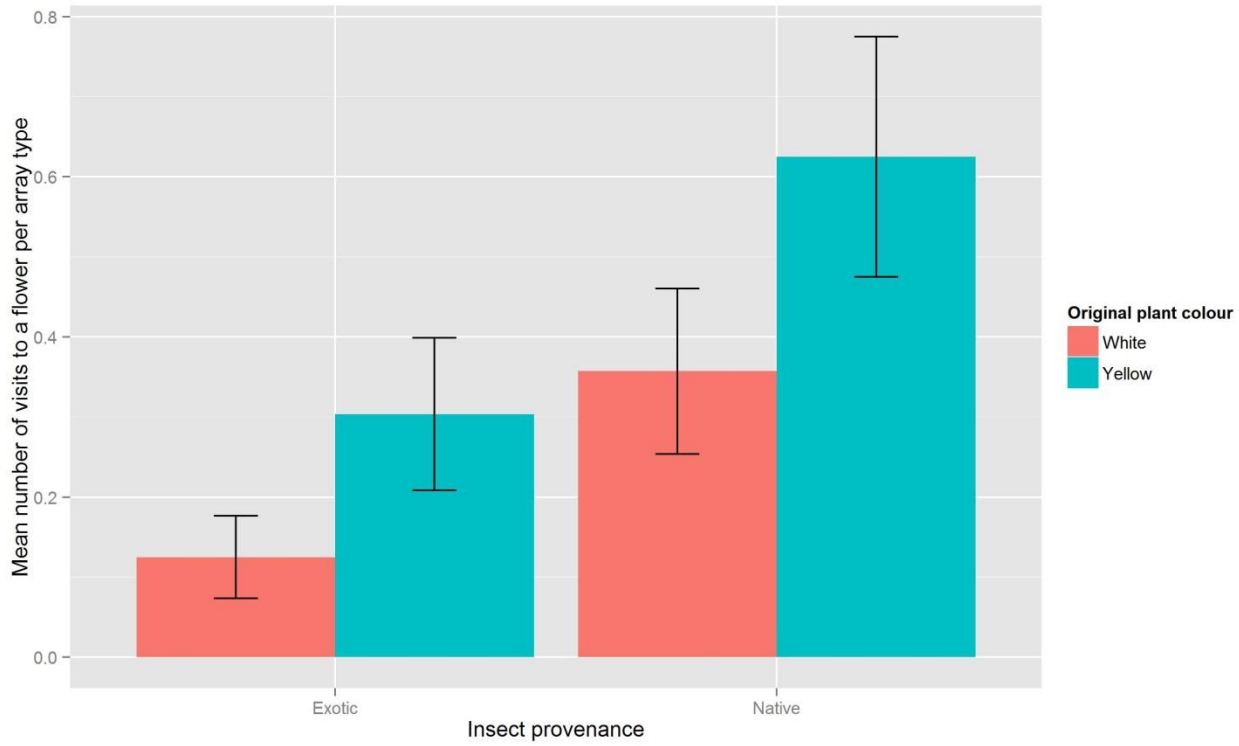

Figure 3.5:: Mean number of visits to a white or yellow flower painted yellow (+SEM) by native and exotic insects at the Christchurch Botanical Gardens.

3.4 Discussion

The colour preferences of New Zealand's flower visiting insects

I did not find a significant effect of yellow or white flower colour on the visitation of New Zealand's invertebrate floral visitors, when visitors were grouped according to their native or exotic status. Pollinator preference for native or exotic flowers was also not significant, nor did it interact significantly with flower colour. These results are unexpected considering the results of the previous chapter and may have been a result of the experimental set up or lack of pollinator visitation across a range of species. This does fit with predictions relating to native flower visitors alone, in that it supports traditional views that New Zealand's flower visitors lack strong colour preferences (Lloyd 1985). Pollinators may have preferred familiar traits as was found by Campbell *et al.* (2010) who found that native alpine insect pollinators often preferred the familiar set of floral traits when flowers were painted. In this chapter, yellow *Helenium flexuosum* flowers painted yellow received more visits to a flower on average per array than white *Pachystegia insignis* flowers painted yellow.

While I was unable to show a significant effect of white or yellow flowers on the visitation of New Zealand's insect visitors this does not mean that a lack of colour preference exists as analysis in this chapter had low statistical power. Therefore I was unable to contribute to explanations of why New Zealand has an unusually high proportion of white flowers and a relative lack of bright blues, reds, and purples (Lloyd 1985). It is possible that an indirect selection pressure is also driving white colouration (explored further in Chapter 5), leading to a greater frequency of white flowers. If native insect pollinators do snub brighter floral colourations then this may allow some protection from mutualist-dependent brightly coloured invasives.

I was unable to show in this chapter that New Zealand's pollinators discriminate between yellow and white flowers, native or introduced plants, or a combination of the two. Because of low statistical power this chapter has been unable to confidently decouple the preferences of pollinators for particular plant attributes further than that which was achieved by the previous chapter. The lack of preference demonstrated here may have been the result of using only white and yellow flowers of a few plant species. Campbell *et al.* (2010) found that *Hylaeus* taxa showed colour preferences when visiting some flower species but not others. As well as this, in their (2010) study *Lasioglossum* taxa did not show a preference for flower colours which is supported by my work in Chapter 2. This is important to note because the large majority of visits in my experiment were made by this taxa. Therefore because insect species were grouped by provenance to increase sample size *Lasioglossum* may have skewed the results of my study as they were overrepresented in the data. Another possible limitation of this experiment is that of the colours included, determined mostly because of time constraints and flower availability. *Bombus* taxa have been previously shown to prefer purple coloured flowers (Wesselingh & Arnold 2000), and *Apis mellifera* and *Bombus* species preferred purple over white flowers in Chapter 2, therefore the exclusion of purple may have impacted their lack of visitation. In the previous chapter the colour preferences of the insect species tested were changed when including yellow and white flowers only, with a significant proportion of the insect species not showing any preference between the two. Also possibly contributing was the use of a yellow native cultivar rather than an uncultivated species, a decision forced by lack of plant availability. However, despite the lack of significant results, it was useful to test insect visitation to different coloured flowers experimentally in an attempt to extend on results found in the previous chapter.

Other possible drivers of pollinator preferences

The association between colour and reward

Understanding the colour preferences of New Zealand's insect fauna is important because they may facilitate or impede floral invasions, provide possible valuable alternative pollination services, and have been a strong selective force in the New Zealand flora through coevolution. The applications to invasion biology and alternative pollinators are explored in the previous chapter and so will not be repeated here. The importance of coevolution between flower visitors and their plants in directing the current New Zealand flora is uncertain. It is likely that white colouration is driven in part by pollinator preferences, and also in part by less direct factors such as plant thermoregulation, water retention, or pleiotropic genes (see Chapter 5). Petal temperature can drive pollinator preferences offshore as some bees associate warmth with certain floral colourations (Dyer *et al.* 2006). In this

idea, bees are also treating heat as a reward, along with the typical rewards of pollen and nectar. Dyer *et al.* (2006) showed that *Bombus terrestris* will make foraging decisions based on the warmth of the flower; warmer flowers aid in their thermal regulation and reduce foraging costs through heat loss. They also demonstrated that these bees were then able to learn to associate certain colours with certain temperatures, therefore using the petal colour as a cue. Therefore the arrival of exotic pollinating insects may change the common colours seen in New Zealand's flora if their visitation is also driven by heat rewards. Because of the complexity in decoupling mutualistic relationships, all possible drivers of decisions made by pollinators must be examined.

It is possible that a preference for another floral trait was driving pollinator visitation in this study as floral morphology (Fig. 3.1), scent, and phenology were unable to be accounted for. While most of the species used here exhibited radial symmetry, *Hebe salicifolia* and did not. As mentioned in the introduction of this chapter and concluded in the last, a number of phenotypic traits may be solely or jointly driving pollinator preferences. These can include pollinator traits such as vision or flower constancy (Gegear & Laverty 2001; Chittka *et al.* 2003; Dyer *et al.* 2012), and floral traits such as colouration; scent; size; and shape (Raguso 2001; Weiss 2001; Campbell *et al.* 2012; Hirota *et al.* 2012; Kaczorowski *et al.* 2012). Size and shape are particular morphological traits that are often tightly linked and difficult to decouple which may be important in driving pollinator visitation.

Other traits may be preferred by pollinators

The size and shape of floral displays may also play a role in pollinator choice; although as with many investigations into phenotypic preferences it is not always easy to distinguish between the two. Results are also often varied across species, and within studies. The nocturnal hawkmoth, *Manduca sexta,* has been shown to have some preference in regards to the shape of artificially constructed *Nicotiana* flowers' corolla at Cornell University, however the authors here suggested that overall floral size may be more prescriptive of preference (Kaczorowski *et al.* 2012). Other studies have been unable to demonstrate a preference for larger corolla widths in the field, and suggest that these may be selected for simply because wider corollas allow a greater pollen export (Campbell *et al.* 1991). Campbell *et al.'*s (2012) study (see introduction, this chapter) also looked at flower size as an indicator of pollinator preference in alpine New Zealand. In their study, larger than average flowers received more visits and this was independent of the colour of the flower. As with other traits, the factors surrounding corolla shape and size are difficult to isolate because of regular correlations between the two. Because morphology can delve into the controversial works surrounding floral symmetry, care must be taken in making any remarks regarding pollinator preference. Long-term experimental work is needed to truly isolate the many traits surrounding morphology such as size, shape, symmetry, and scale, before any substantial statements about pollinator preferences can be made. Indirect factors are also likely important, although much harder to test.

It is often difficult to isolate the traits that are driving pollinator choice, because a number of traits may be interacting. Often in the absence of one trait another may suffice, even if this may not normally be the strongest driver of pollinator choice. Scent does not appear to feature as frequently as the main driver of pollinator choice, however, for beetles it can be a major determining factor in the absence of other cues (Weiss 2001). Similarly, flies seem to be driven by visual cues when distantly foraging but switch to olfactory cues as they approach; and in deceptive floral species scent

appears to be the predominant cue to pollinators (Weiss 2001). Following the claims of Weiss (2001) and Raguso (2001) which state that hawkmoths tend to rely on scent in making distant foraging decisions, a Japanese study compared the response of swallowtail butterflies and nocturnal hawkmoths to changes in scent, colour, or both scent and colour in *Hermerocallis fulva* and *Hermerocallis cirtina* hybrids. Both pollinating species appeared to have colour preferences in their visitation frequency, but no particular preference for scent (Hirota *et al.* 2012). This finding is supported by an investigation into an epiphytic orchid, *Tolumnia variegate*, in Puerto Rico. There was no evidence in their results to suggest that fragrance phenotypes had a significant correlation with the reproductive success of this species (Ackerman *et al.* 1997). Other preferences may have evolved based on rewards, which then encouraged supernormal stimulus by plants (Chittka & Thomson 2001). One untested example of this is pollen colouration. It has been suggested that pollen was originally coloured yellow as a result of the protection gained from UV radiation. Pollinators may then have developed a preference for yellow signals as a result of the association between yellow and rewards, and plants may then have produced yellow nectar-guides to exploit this preference (Chittka & Thomson 2001). A generalisation may be made that flowers that rely on particular suites of pollinators for reproductive success often use different cues which exploit the particular receptors of their target pollinator; and this generalisation may again lead into the uncertain territory of floral syndromes (Weiss 2001).

Foraging efficiency in insect-pollinator choice

The visual capabilities of pollinators are also important factors when determining floral traits. One study which investigated the legitimacy of floral syndromes, focused on red flowers. It is suggested that flowers are often red to encourage hummingbird visitation and discourage that of bumblebees (Chittka *et al.* 2001). While bumblebees still visit these red flowers, and hummingbirds have not yet been shown to have a noticeable preference for red, a change in only a few percent in plant fitness may drive this colouration (Chittka *et al.* 2001). As to why the flowers are red, Chittka *et al.* (2001) proposed that this colour may increase the search times for bumblebees through their visual capabilities, and thus succeed in deterring them. They investigated this by providing *Bombus terrestris* with three different floral arrangements, and found that red and white arrangements took longer to forage through. This may have perhaps been a result of the difficulty for *Bombus terrestris* in differentiating colours that contrast poorly with their environment (Chittka & Thomson 2001), and demonstrates how forager vision may influence floral evolution.

New Zealand's flower visiting invertebrates may have some preferences in regards to the colour of the plants they visit. While I was unable to show a significant effect of colour on visitation I did not show that they do not have a preference. If the preferences of pollinating insects in New Zealand are determined the direct drivers of the common traits in the New Zealand flora can be identified, and the level of pollinator specialisation will be better understood. This is important because the degree of specialisation between plants and their pollinators can influence the risk of plant extinctions (Johnson & Steiner 2000). There is still much to be understood about the effect of flower colour on the visitation of New Zealand's pollinating insects.

Chapter 4

A case study: Does *Gastrodia* 'long column' rely upon mutualisms for successful fruit set?

4.1 Introduction

The reproductive systems of New Zealand's orchids are poorly understood (Lehnebach *et al.* 2005) and in order to protect threatened orchid species we need to address this lack of understanding. Orchids are one of the largest angiosperm families worldwide with huge diversity across form and function. The orchid species which rely upon animal vectors for reproduction include rewarding and deceptive species. There are over 100 species of orchid in New Zealand, and the majority of these are terrestrial (Lehnebach *et al.* 2005). These terrestrial orchids are often threatened by habitat changes or losses; however it is unknown whether this group is also vulnerable to breakdowns in mutualistic relationships with pollinators (Lehnebach *et al.* 2005). Bond (1994) states that to assess the risk of a breakdown in a plant-pollinator mutualistic relationship three things must be considered. These are: the risk of mutualism failure, the level of dependence on the mutualism for successful reproduction, and the quantifiable dependence of a population on seed production. Therefore it is necessary to extend current knowledge of the dependence of orchids on mutualistic services for reproductive success. Here I focus my attention on the undescribed *Gastrodia* 'long column'. *Gastrodia* is a small genus of over 50 species (Yeh *et al.* 2011), and the New Zealand *Gastrodia* taxa are identified by their column length, colouration of the apex of the labellum, and the texture of the perianth (Rolfe 2010). The Rolfe (2010) guide will be used in the identification of *Gastrodia* 'long column' in my study. This chapter serves to determine the importance of pollinators in the fruit production of *Gastrodia* 'long column'.

An overview of the Orchidaceae

Orchids are monocot angiosperms, and are one of the largest angiosperm families in the world (Johns & Molloy 1983) with somewhere from 17,000 to 35,000 species (Dressler 1993). There are some structural features which are common in this group such as flower parts which come in sets of three (for example three petals and three sepals), and within the flower lies a column (Johns & Molloy 1983). The 'column' is made up of the (at least partially) fused stamen and pistil (male and female parts), with the stamen found on only one side of the flower (Johns & Molloy 1983). Pollen clumps together to form 'pollinia' and fertilised flowers tend to produce small seeds in great numbers (Johns & Molloy 1983). Flowers can last anywhere from single days to many weeks, and flowers vary in size and colour from minute to large, dull to colourful (Johns & Molloy 1983), and simple to complex (Dressler 1993). Approximately 73 % of orchids are epiphytic, with plant-pollinator relationships ranging from specialised to generalised (Dressler 1993). A review of Orchidaceae from southern Africa revealed a significant proportion of these species were highly specialised in their plant-pollinator relationships, to the point that some plants were pollinated by a single species (Johnson & Steiner 2003). Johnson and Steiner (2000, 2003) noted a trend for specialisation of orchid systems in the tropics and temperate zones of the southern hemisphere, and

a trend for generalisation in pollination systems for the northern hemisphere. These trends may serve as useful preliminary predictors when studies are conducted.

Reproduction systems of orchid plants

The reproductive systems of orchids are diverse, from highly complex to comparatively simple. Reproductive barriers can occur at the pre- and post- pollination stages and may include cues which influence which pollinators visit. A few possible such cues are scent (Pellegrino *et al.* 2012), morphology (Pedron *et al.* 2012), colour, and phenology (Peter & Johnson 2013). Autogamous species such as *Cyrtosia septentrionalis,* which have bright displays to attract pollinators, are often not pollen limited under natural conditions despite a lack of pollinators because of their ability to self-pollinate when necessary (Suetsugu 2013). This is achieved by allowing contact between the stigma and pollinia (Suetsugu 2013) which is important because such abilities to self-pollinate need be considered when considering the risk of failure of a mutualistic relationship.

While studies which focus on orchid pollination are increasing, published works are still few (Pedersen *et al.* 2013). Orchid pollination is diverse and many plants deceive their pollinators (Dressler 1993). A Japanese study showed that one way in which a pollinator dependent orchid species may ensure pollination while not providing nectar rewards is through seasonal timing (Sugiura 2013). It is thought that *Calanthe striata* ensures pollination by carpenter bees through early blooming resulting in visitation by naïve bees. This strategy also reduces competition as other reward-providing floral species are not readily available (Sugiura 2013). Orchids can also be in mutualistic relationships by providing food rewards for their pollinators. This is seen in the four *Habenaria* species; *H. megapotamensis, H. johannensis, H. macronectar,* and *H. montevidensis*; which have been shown to provide nectar rewards to their butterfly and hawkmoth pollinators in the State of Rio Grande do Sul, southern Brazil (Pedron *et al.* 2012). Plants may also provide atypical rewards which can initially appear deceiving without closer inspection. An interesting example of the unusual structures found in pollination systems of the Orchidaceae is that of *Serapias* in the Calabria region (*Serapias lingua* L*., Serapias parviflora* Parl., *Serapias cordigera* L. and *Serapias vomeracea* Briq.) (Pellegrino *et al.* 2012). *Serapias* flowers angle towards the ground and form a small tube which can provide shelter from stressful conditions or nests for potential pollinators (i.e. solitary bees), thereby receiving visitation and possibly providing another kind of reward (Pellegrino *et al.* 2012). Therefore these three examples demonstrate the variability in the relationships orchids have with their pollinators.

Also important to note when considering orchid pollination generally is that of the difference in success of rewarding and non-rewarding species. Orchid species with nectar rewards have been shown to have a higher fruit set than those without (Neiland & Wilcock 1998; Smithson 2009). A review of Orchidaceae pollinated by carpenter bees found that flowers typically exhibit light to dark pink petals with a shallow corolla, with the low reproductive success rate common to deceptive floral species (Sugiura 2013). Further, Johnson and Nilsson (1999) found that artificially increasing the nectar rewards of *Orchis mascula* and *Orchis morio* led to more frequent and prolonged visitation by queen bumble bees. This Swedish study (on the island of Öland just off the coast of Sweden) hypothesized that increased nectar rewards would lead to an increase in geitonogamy although their data did not support this (Johnson & Nilsson 1999). Nectar rewards are not

straightforward. While nectariferous species produce twice the fruits on average, those in the tropics have a very low fruit set regardless of their nectar providing status (Neiland & Wilcock 1998) and in the Swedish study above increased nectar rewards did not lead to increased fruit set in *Orchis morio* (Johnson & Nilsson 1999). Rewarding and non-rewarding species may have different reproductive success, and they use a number of different strategies to prevent pollination limitation.

Cheating in Orchidaceae

Because a significant proportion of the Orchidaceae cheat (at least a quarter) (Dressler 1993; Johnson & Nilsson 1999; Smithson 2009), I will go into this strategy in more detail.

In a mutualistic plant-pollinator relationship a plant gains pollen transfer, while a pollinator gains a meal or another resource (Smithson 2009). Cheating in plant-pollinator interactions has two forms; cheating by plants, and cheating by pollinators (Smithson 2009). Cheating by pollinators is done through nectar or pollen removal without providing a pollination service to the reward-providing plant (Smithson 2009). Pollinators possibly have a greater capacity for cheating because of their mobility and freedom of choice. While cheating is often regarded as detrimental to the plant, some studies have demonstrated this to have a neutral effect, while others have demonstrated beneficial effects through pollination or changes in fellow pollinator behaviours (Maloof & Inouye 2000; Smithson 2009). Nectar robbing warrants mention as this can have a particular impact on species and is considered reasonably common amongst orchid species. Nectar is often 'robbed' by a pollinator (i.e. a bumblebee) biting through the corolla of a flower and accessing the rewards (Maloof & Inouye 2000). Other pollinators have small smooth bodies that pollen fails to stick to and so here insects may be paid for services not rendered (Osborne & Free 2003). Pollinators are opportunistic, and both parties optimise their own fitness without regard for the other party's interests (Smithson 2009).

Plants can also cheat by deceiving pollinators into visiting their flowers without providing any reward, a process controversial among evolutionary biologists (Smithson 2009). It has been suggested for the Orchidaceae clade that mutualisms have evolved repeatedly from cheating (Smithson 2009). One method of orchid cheating is that of sexual deception (Pellegrino *et al.* 2012; Rakosy *et al.* 2012; Vereecken *et al.* 2012). This is demonstrated in the genus *Ophrys* where the labellum of the deceiving flower imitates females of the bee pollinating species, and the flower's scent imitates that of sex pheromones (Schiestl *et al.* 1999). In this way it successfully attracts pollinators intending to mate without providing rewards for their pollinator service. Other orchid species which use sexual deception to cheat are *Drakaea livida* and *Caladenia pectinata* (Phillips *et al.* 2013). These two orchid species have different flower morphologies but both use sexual deception to attract the male wasp *Zaspilothynnis nigripes* in remnant bush land adjacent to Ruabon Nature Reserve, south of Perth, Western Australia (Phillips *et al.* 2013). It is unusual that both orchid species share the same wasp in such a specialised way, and suggests that morphological flower traits are perhaps not the strongest driver of pollinator attraction in these orchid species (Phillips *et al.* 2013). The pollination success of orchids which use deception is expected to be low, although species such as *Calypso bulbosa* have been shown to have up to 60% fruit set despite their deceptive pollination system (Abeli *et al.* 2013). It is thought that frequency-dependent selection will prevent mimicking taxa from exceeding the frequency at which pollinators learn to discriminate against

these cheating species. Cheating appears to be a fixed strategy in these particular taxa, with plants achieving general rather than specific mimicry of particular rewarding plant species (Smithson 2009). Sexually deceptive plants are less restricted by frequency because male wasps cannot learn to ignore female pheromones because their fitness would be drastically reduced. Therefore plants that used sexual mimicry and rewarding-plant mimicry are under different selection pressures. Deceptive species are common across the Orchidaceae.

Insect pollinators in New Zealand

New Zealand's insect pollinators

New Zealand has a range of invertebrates which perform pollinating services (see Chapter 3). Of these the main groups are bees (Hymenoptera), flies (Diptera), butterflies, and moths (Lepidoptera) (Newstrom & Robertson 2005; Donovan 2007). Many of these species' visitation is relatively generalised and therefore they visit a wide range of source plants (Lloyd 1985; Donovan 2007). Flies are often important when other pollinators are inactive, such as in colder months or alpine areas (Newstrom & Robertson 2005). Exotic pollinators such as bees also contribute to pollination, most of which were introduced to New Zealand deliberately for crop pollination (Howlett & Donovan 2010).

Native pollinators: Lasioglossum *bees*

The native insect pollinating species in New Zealand are thought to be reasonably generalised in the flowers they visit (Lloyd 1985; Newstrom & Robertson 2005). Some of the most important pollinating insects in New Zealand are the bees (Hymenoptera), many of which are endemic. All of New Zealand's native bees belong to either the Colletidae (Newstrom & Robertson 2005; Donovan 2007) or Halictidae (Donovan 1980, 2007). *Lasioglossum* bees (family: Halictidae) are the only bees in the subfamily Halictinae found in New Zealand (Donovan 2007). There are four species which have been identified in New Zealand; three from the subgenus *Austrevylaeus: Lasioglossum mataroa, Lasioglossum maunga, and Lasioglossum sordidum;* and one from the subgenus *Chilalictus: Lasioglossum cognatum* (Donovan 2007). Of these four species, *Lasioglossum cognatum (*a species shared with Australia) has been seen visiting the smallest range of native and exotic plant species, while *Lasioglossum sordidum* visits a wide range. The ability of *Lasioglossum sordidum* to visit a broad range of native and exotic plants is thought to account for its abundance. At many sites this species outnumbers other native solitary bees throughout the year, and is a successful competitor of *Apis mellifera* (Donovan 2007). All of these *Lasioglossum* species nest in the ground and are found in both the North and South Island of New Zealand, although *Lasioglossum maunga* is primarily a montane species. These bees are important as pollinators in New Zealand because of both their abundance and their lack of plant specialisation.

New Zealand orchids

There are 25 genera of orchid in New Zealand which incorporate over 100 different species (Lehnebach *et al.* 2005). Of these species more than 90 are terrestrial (the most threatened of the New Zealand orchid species), and a large proportion of the terrestrial genera contain only one species (Lehnebach *et al.* 2005). Although flowers in New Zealand are often smaller than orchids

elsewhere in the world this is consistent with all orchids native to a temperate climate; particularly those with a depauperate pollinating fauna (Johns & Molloy 1983). One genus which demonstrates the diversity of reproduction of New Zealand orchids is *Pterostylis* (Johns & Molloy 1983). This genus has many species that self-pollinate, but also a number of species which rely on insect vectors (Lehnebach *et al.* 2005). *Pterostylis* uses an unusual method for insect pollination: the orchid traps an insect inside the flower using a mechanical hinge (Johns & Molloy 1983). This forces the insect to move through a narrow tunnel to escape and ensures pollen is collected or deposited successfully (Lehnebach *et al.* 2005).

Orchid pollination in New Zealand

In New Zealand, research on the pollination of orchids is limited (Lehnebach *et al.* 2005). However, it is estimated that more than 60% of the orchids in New Zealand have a self-pollinating breeding system (Molloy 1990) and insect-dependent species have also been observed (Lehnebach *et al.* 2005). For example, a study which looked at the breeding systems of four New Zealand epiphytic orchids found a range of results. Two species, *Earina autumnalis* and *Earina mucronata* were found to be self-incompatible, while the remaining two species *Earina aestivalis* and *Winikia cunninghamii* had at least low levels of self-compatibility (Lehnebach & Robertson 2004). Interestingly, all four species were seen to be dependent upon insect vectors for successful fruit set, with a range of fly and bee visitors observed visiting their flowers (Lehnebach & Robertson 2004). Both native and exotic insect pollinators have been observed visiting these four native orchids including *Apis mellifera, Bombus terrestris, Eristalis tenax, Melangyna novaezelandiae, Melanostoma fasciatum, Dilophus nigrostigmus,* and *Helophilus antipodus,* and this system appears very generalist (Lehnebach & Robertson 2004). Another study has observed aphids visiting *Gastrodia cunninghamii* and *Zygomyia* (fungus gnats) upon *Pterostylis alobula* (Lehnebach *et al.* 2005). Therefore, while little is known about orchid pollination in New Zealand, it is likely that insects play a significant role in the pollination of many species.

The role of thrips and aphids in orchid pollination

The role of very small insects in pollination (i.e. thrips and aphids) has received little attention, particularly in New Zealand. Thrips (Thysanoptera) in particular, are usually thought either inconsequential or to act as pests, however it has been suggested that they may play an undocumented role in pollination (Norton 1984; Newstrom & Robertson 2005). While a single individual is only able to transport a few pollen grains, their populations grow to such a size that this transfer may be significant (Newstrom & Robertson 2005). The possibility of thrips as effective pollinators was examined in 13 species of tree from lowland forest of New Zealand (Norton 1984). While this study was unable to draw firm conclusions due to a lack of data, circumstantial evidence implied that *Thrips obscuratus* were possibly effective pollinators because of the breeding systems of the plants, and the frequency of *Thrips obscuratus* visitation (Norton 1984). Aphids and thrips have also been seen to perform some pollination service in other species worldwide such as herbs in north-western Iowa (Baker & Cruden 1991). Thrips and aphids were observed with pollen on both of these two herbaceous species (*Ranunculus sceleratus* and *Potentilla rivalis*) which were known to outcross (the authors observed bee pollination occurring) (Baker & Cruden 1991). Secondly, a southeast Asian study showed that thrips are the main pollinators of mast-fruiting dipterocarp trees

(Ashton *et al.* 1988). Here 95% of flower visitors to the highly self-incompatible *Shorea* species, in Pasoh Forest Reserve in western peninsular Malaysia, are from either the *Thrips* or *Lemurothrips* genus (Ashton *et al.* 1988). Pollen of these trees is sticky and rarely moved by wind and the bodies of the thrips visiting these trees were covered in *Shorea* pollen. Movement between trees was usually achieved as thrips fell and were transported by wind currents (Ashton *et al.* 1988). Therefore despite their small size thrips can be important in the pollination of some species, and perhaps their possible importance can be hypothesized by pollen morphology. Thrips and aphids are mentioned because the authors of a study which included *Gastrodia cunninghamii* believed that aphids may have played some role in *Gastrodia cunninghamii's* pollination and this species is closely related to *Gastrodia* 'long column' (Lehnebach *et al.* 2005).

The *Gastrodia* orchids

Gastrodia species are commonly known as the potato orchids (Scanlen & St George 2011). These plants are leafless, non-photosynthetic, saprophytic orchids with spirally arranged flowers, and most species are found in Asia or Australasia (Dressler 1993). There are believed to be over 50 *Gastrodia* species which can be found in Africa, Asia, and Oceania; and new species are frequently being added as this genus becomes more widely studied (Yeh *et al.* 2011). Worldwide, difficulties in understanding the breeding systems of *Gastrodia* are common. A study of *Gastrodia exilis* in Thailand did not observe any insect visitors to these flowers and found no difference in the seed sets of bagged (mesh) and open (not bagged) plants, despite a floral morphology which suggested that autonomous selfing was unlikely (Pedersen *et al.* 2005). The authors here suggest that minute insects such as thrips may play some role, which is consistent with Lehnebach *et al.*'s (2005) suggestion of aphids (or thrips) playing an as yet unrecognised role in *Gastrodia cunninghamii's* pollination. Another study reported sighting an *Exoneura* bee pollinating *Gastrodia sesamoides*, however, this work is only mentioned in passing (Dressler 1993). This illustrates the lack of literature on the breeding systems of *Gastrodia* worldwide.

Gastrodia *orchids in New Zealand*

There are 15 *Gastrodia* species in Australasia (Lehnebach *et al.* 2005) and five *Gastrodia* taxa in New Zealand (Scanlen & St George 2011), and some work on *Gastrodia* pollination has been published. A study which looked at *Gastrodia cunninghamii* in the North Island of New Zealand over a 2 month long flowering period had conflicting results when examining *Gastrodia cunninghamii's* breeding system. Seed production of *Gastrodia cunninghamii* failed following self-pollination by hand, but seeds were produced following both autonomous selfing and outcrossing performed by hand (Lehnebach *et al.* 2005). Both results (successful outcrossing and the failure of *Gastrodia cunninghamii* to set seeds following hand-pollinated selfing) are unusual because this species has long been thought to be autonomously selfing (Lehnebach *et al.* 2005). The failure to set fruits following hand selfing but success autonomously suggests methodological errors in their study. Contributing to the conflicting evidence surrounding this orchid's dependence upon pollinators; *Gastrodia cunninghamii* was found to have no discernible scent, although stains taken indicated the potential for scent production (Lehnebach *et al.* 2005). Aphids were observed within *Gastrodia cunninghamii* flowers, allowing for the possibility of some role in pollination (Lehnebach *et al.* 2005) although their study did not pursue this further. Because of the apparent selfing system of

Gastrodia cunninghamii (unusual results were thought to be methodical errors) this study concluded that this species is less vulnerable to mutualistic breakdowns. However, conversely, a selfing species may be more at greater risk of changing environments because of its lower genetic variability (Lehnebach *et al.* 2005).

Chapter objectives

As mentioned in the first paragraph of this chapter, Bond's (1994) criteria for assessing the risk of plant extinctions has three aspects; probability of pollination failure, dependence up the mutualism for reproductive success, and a plant's dependence upon seeds. Here I look at *Gastrodia '*long column's' dependence upon mutualistic services for successful fruit set. This can contribute to future projections about its risk of decline. My study was designed when a large number of native *Lasioglossum* bees were observed visiting open *Gastrodia* 'long column' flowers (See Fig. 4.1) in central Christchurch. The bees were noted to have pollinia stuck to a number of different body parts (back, side, legs), and appeared very interested in trying to groom them off. Indeed the only limiting factor in the number of pollinia being transported was seemingly the number of available flowers with some pollinia remaining. I therefore set up a study to test the dependence of *Gastrodia* 'long column' upon mutualists for successful fruit set.

The specific research questions are:

1. Is *Gastrodia* 'long column' visited by insect pollinators?
2. Does *Gastrodia* 'long column' rely on animal pollinators to set fruits?

4.2 Methods

Study species and sites

The focal species of this study is an undescribed entity which has been given the tag name of *Gastrodia* 'long column' (Rolfe 2010). This plant is identified by: a distinctive labellum which has a yellow tip and under ridges, and a longish column as can be seen in the figure below (Fig. 4.1) (Rolfe 2010). These flowers are said to be faintly scented (Scanlen & St George 2011), but I was unable to detect any scent. Plants are usually found south of Owhango (central North Island) down to parts of Steward Island, in cooler locations beneath trees and shrubs (Scanlen & St George 2011). *Gastrodia* 'long column' is often mistaken for *Gastrodia* 'long column black', *Gastrodia* aff. *sesamoides,* or *Gastrodia cunninghamii,* which are very similar with slight phenotypic and temporal differentiations. *Gastrodia* 'long column black' has a black labellum tip, *Gastrodia* aff. *sesamoides* has creamy flowers which bloom slightly earlier in the season, and *Gastrodia cunninghamii* has a dark labellum tip and a much shorter column (Scanlen & St George 2011).

Data were collected from a residential property in central Christchurch (6 Beveridge Street). This address is approximately 1.5km from the site at the Christchurch Botanical Gardens described in later chapters. While it would have been preferable to use the Christchurch Botanical Gardens for this study (and *Gastrodia* 'long column' was flowering there), the *Gastrodia* plants found in the Christchurch Botanical Gardens had all been uprooted from the ground when I intended to commence my experiment, possibly by wind. *Gastrodia* plants in this urban location were all located

in the same section of garden, approximately in a 1x1 m square. This area is highly urbanised with the garden located next to a driveway, therefore plants were adjacent to pedestrians and vehicles which may have impacted pollinator visitation or plant health to some extent. The *Gastrodia* plants were growing under a hedge of *Coprosma robusta* and *Pittosporum tenuifolium,* on a spot which had previously hosted a red beech tree which had died in 2002.

Insect observations and bagging

Observations began on 29 January 2012 and finished on 6 February 2012. These observations were instantaneous which I defined as the total counted number of flower visitors resting upon flowers in one instance per stalk. I counted all flying invertebrate visitors observed on flowers of my 11 stalks. Most visits were by *Lasioglossum* bees. Aphids were observed on pollinia, but were not counted because of their small size, the difficulties in excluding them following bagging, and uncertainty surrounding their ability to move pollinia (Fig. 4.1). Flower visitors were defined as all flying insects which were on focal flowers when counted, and no assumption based upon pollen transfer or effectiveness was made.

Figure 4.1: An aphid upon a pollinium which is stuck to the back of a native *Lasioglossum* bee, urban Christchurch, 28 January 2012, photo by D. Kelly.

Some flowers were bagged to exclude pollinators to determine whether there was a significant difference in fruit set depending on whether pollinators could or could not visit flowers (and therefore provide a pollination service). Because this experiment began so late in the season only three stalks had a sufficient number of unopened flowers available for bagging. Plant stalks were

tagged and the total number of flowers (living, aborted, and unopened-bagged), were counted on all stalks. Bags were attached to the three inflorescences with unopened buds still available, with 11 *Gastrodia* stalks in total tagged. Bag attachments, tagging, and general preliminary work were all conducted on 30 January 2012. This was a warm sunny day (~20°), with light winds (1.5 ms^{-1}) and a cloudless sky. Bags used were light nylon**,** attached gently with ties. All observations were conducted under relatively warm (>15°), sunny conditions. Photos were taken of unknown insects in the field and later identified using *Which New Zealand Insect?* By Andrew Crowe, and *A Photographic Guide to Insects of New Zealand* by Brian Parkinson. Final fruit set was recorded on 16 February 2012 (two weeks after bags were added) by counting all fruits on the eleven stalks and comparing with the original flower counts. This allowed me to determine whether the bags (and therefore pollinator exclusion) affected fruit set. It should be noted that the bag on stalk 3 was knocked off sometime between 4 February and 6 February and replaced upon discovery on 6 February, which may be why this plant has higher fruiting success than plants 2 and 4.

Statistical analysis

Data were initially examined with Chi Squares tests using the program Statistix (Version 8 by Analytical Software Ltd) to determine whether there was a relationship between the proportion of flowers setting fruit and the treatment received (bagged or open flowers) on bagged stalks only. To further test whether there was a significant reduction in fruit production following pollinator exclusion I used a binomial generalised linear mixed model with a random term for plant stalk (version 2.15.0 R Foundation for Statistical Computing 2012). This allowed me to account for any possible background variation caused by the different stalk individuals and to include all stalks in analysis.

The binomial model used was:

```
model <- lmer(cbind(fruit, flowers-fruit) ~ bagged + (1|stalk), family=
binomial, data = data1)
```

4.3 Results

Observations yielded 37 insect visits over a week long period (. Flower visitors to *Gastrodia* 'long column' over this period included two taxa of native solitary bee; *Hylaeus* and *Lasioglossum*, and two Diptera; *Parentia* and *Melangyna novaezelandiae.* All of these visitors were seen on the flowers of *Gastrodia* stalks. Visitation was primarily done by *Lasioglossum* (Fig. 4.1) which made up 31 of the 37 visits (~84%). All buds were no longer receptive by 6 February 2012 when observations stopped, and fruits were all set by 16 February 2012 which was when the final count was taken. A number of *Parentia* and *Hylaeus* species were observed flying around the stalks following bagging. This shift towards *Hylaeus* is consistent with the apparent seasonality of their abundance which is noticed in later chapters, where *Lasioglossum* appeared earlier and *Hylaeus* did not become common until later in the summer.

Figure 4.2: *Lasioglossum* bee with pollen collected on its legs and a pollinium stuck to its back on flowers of *Gastrodia* 'long column' in urban Christchurch, 28 January 2012, photo by D. Kelly

Lasioglossum bees were the key pollinator during observations and were seen entering flowers and leaving with pollinia attached (Fig. 4.2). Open flowers had high fruit set; therefore there are enough *Lasioglossum* bees in an urban area for pollination of this plant species. Additionally these stalks did not get a high fruit set when autonomously selfing as only ~15% of the bagged flowers set fruit, therefore *Gastrodia* 'long column' depended upon *Lasioglossum* bees for successful reproduction in urban Christchurch (Table 4.1).

Table 4.1: Fruits, bagged and open flowers, and the percentage of fruit set of 11 *Gastrodia* 'long column' stalks at a residential property in central Christchurch.

Treatment	Stalk	Flowers	Fruits	% fruit set
Not bagged	8	182	156	85.7
Bagged	3	13	2	15.4
Open flower on bagged stalk	3	97	54	57.7

A significant dependence upon pollinators for successful fruit set was detected. As can be seen above (Table 4.1) there was a significant loss of fruit set following the bagging of flowers for the three stalks which received a bagging treatment (plants 2, 3, and 4). In addition, fruit set was normally quite high, with untreated stalks having over 80% success (Table 4.1). This was significant when tested using a two way chi-square test for independence of treated stalks (Table 4.2 and Fig. 4.3, P=0.02, 1df).

Table 4.2: The observed and expected values (in brackets) of bagged and open flowers of *Gastrodia* 'long column' on plants which received a bagging treatment using 1 df.

	Failed	Fruit
Bagged	11 (6.38)	2 (6.62)
Open	43 (47.62)	54 (49.38)

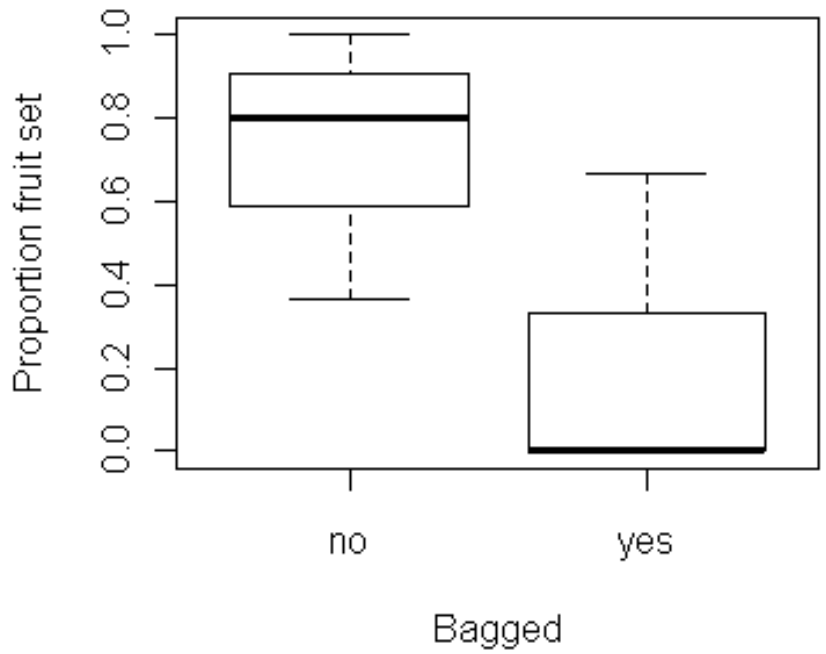

Figure 4.3: Box plot of the proportion of fruit set for bagged and open flowers of *Gastrodia* 'long column' on stalks which received a bagging treatment.

Fruit set was still significantly correlated with whether a flower had been bagged or not for all stalks when accounting for the natural variation between plant individuals included in the study (P=0.02, z= -2.36) (Table 4.3).

Table 4.3: Parameter estimates, standard error, z statistics, and *P*-values of a binomial mixed model (GLMM) testing the effects of plant bagging on fruit set. Stalks were included as a random effect.

Fixed Effects	Estimate	Std. Error	z Value	Pr (>|z|)
Intercept	1.45	0.40	3.27	<0.001
Bagged - yes	-1.94	0.82	-2.36	0.02

4.4 Discussion

This is both the first report of *Lasioglossum* bees visiting *Gastrodia* 'long column' and removing its pollinia, and the first report of *Gastrodia* 'long column's' dependence upon pollinators for successful fruit set.

The importance of mutualistic services to *Gastrodia* 'long column'

Bond (1994) states that when assessing the risk of plant extinctions the dependence of the focal plant upon mutualistic services must be determined. While most of New Zealand's tested native terrestrial orchids follow a selfing breeding system, there have been a few publications which establish that insect-reliant species are also present (Lehnebach *et al.* 2005). However, as so few species have been tested it is still unknown which breeding system is more common. Understanding the breeding systems of plants is important when assessing extinction risk because they show how many options for successful pollination a plant has under conditions of low pollinator abundance (Bond 1994). I have shown that *Gastrodia* 'long column' relies strongly upon pollinators such as *Lasioglossum* for successful fruit set. *Gastrodia* species in New Zealand are an interesting study system because so little is known about their reproduction. The mutualistic reproductive system of *Gastrodia* 'long column' is particularly interesting because orchid species are often deceptive (see the introduction of this chapter), and this species' inconspicuous, dull appearance may at first suggest a self-pollinating system. However, native *Lasioglossum* bees were the predominant visitor to this species over the week of observations. Therefore *Lasioglossum* bees were moving the pollinia of *Gastrodia* 'long column', and *Gastrodia* was dependent upon this transfer for successful fruit set.

Some other orchid species in New Zealand such as *Winika cunninghamii* depend entirely upon pollinators for fruit set because they are not capable of autonomous selfing (Lehnebach & Robertson 2004). Other studies which focused on *Gastrodia* species such as *Gastrodia cunninghamii* did not find a significant reduction in seed set following bagging (Lehnebach *et al.* 2005), suggesting that there is great variation in the reproductive systems of orchid species found in New Zealand. The reproduction system of *Gastrodia* 'long column' is more similar to *Winika cunninghamii* than a member of its own genus, *Gastrodia cunninghamii.* Therefore continued investigations into different *Gastrodia* and other orchid species are important in understanding the pollination systems of New Zealand's native plant species.

The insect visitors of *Gastrodia* 'long column'

My week of observations showed regular visitation by native pollinating species, despite the cryptic nature of these plants, and their location under a hedge in urban Christchurch. Considering the urban location of this study site this can be considered remarkable in that it implies that pollinators are present in sufficiently high numbers for pollination in the middle of a New Zealand city. Final fruit sets of open flowers were high with an average of 78% success for unmanipulated stalks. This is similar to the seed production of *Gastrodia cunninghamii*, which was ~92% in a North Island study (Lehnebach *et al.* 2005). It is interesting that native bee species are able to survive in residential properties in high abundances, and this resilience could be expanded on for future knowledge about the pollinating invertebrate fauna of New Zealand.

Lasioglossum *bees* visit Gastrodia *'long column'*

Lasioglossum bees were the main visitor to *Gastrodia* 'long column' flowers and were seen moving pollinia. There are four *Lasioglossum* species in New Zealand. *Lasioglossum sordidum* has been found throughout New Zealand and was the most likely *Lasioglossum* species observed. This bee species is thought to be in abundance across northern and eastern parts of the South Island in any location which has suitable nesting substrate and floral resources (Donovan 2007). The final three *Lasioglossum* species*; Lasioglossum cognatum, Lasioglossum maunga,* and *Lasioglossum mataroa;* were probably not the bees observed. This is because *Lasioglossum cognatum* is thought to be in only northern coastal parts of the South Island and *Lasioglossum maunga* is not found in lowland areas (Donovan 2007). *Lasioglossum mataroa* is less distributed and abundant than *Lasioglossum sordidum* and is found predominantly in dry montane areas of central South Island. It would be interesting to determine where the *Lasioglossum* bees observed in this thesis are nesting, and whether their abundance implies a high resilience to human-driven environmental changes. This is important from a conservation perspective as botanical gardens are often the only form of large green open space seen for several kilometres in urbanised areas, and they may be playing the role of a biotic corridor for migration, as well as providing sufficient habitat for displaced species.

Females of *Lasioglossum sordidum* species have been recorded visiting 71 native plants, while males have been recorded visiting only 33 native plant species (Donovan 2007). These native plants were predominantly white, with a range of shapes and sizes. Introduced plants are approximately equally visited by *Lasioglossum sordidum,* with 68 plant species visited by females and 40 recorded plant species visited by male bees (Donovan 2007). Introduced plants visited are much more varied, with plant species coloured white, yellow, orange, red, and pink; of many different shapes and sizes. Therefore these bees likely lack strong preferences and the frequent visits to native flowers coloured white is probably a result of the high frequency of white plants in New Zealand's native flora rather than a preference for them (Lloyd 1985). The polylectic foraging habits of *Lasioglossum sordidum* suggest that this bee can forage on any plant whose rewards it can gain access to, and it is active for longer periods than the flowering season of many of its host plants. Their small size increases their ability to visit many flowers but prevents them from opening some flowers and their short tongues can prevent them from accessing the nectar of species that are too narrow to enter successfully (Donovan 2007). Therefore while they may be the main pollinator of *Gastrodia* 'long column' in urban Christchurch, they are not in a tight mutualistic relationship with this plant species. The abundance of *Lasioglossum sordidum* across New Zealand and its generalist foraging habits suggest that *Gastrodia* 'long column' is not at risk of pollination failure at this stage.

Aphids as pollinators

It is possible that aphids play an unrecognized role in pollination of some New Zealand plant species (Norton 1984). A previous paper which looked at *Gastrodia cunninghamii* observed aphids within the flowers of this species (Lehnebach *et al.* 2005), and this is interesting to note as my study observed an aphid sitting on a pollinium of *Gastrodia* 'long column' although an ability to move these pollinia has not been tested (Fig. 4.3). The reliance upon aphids to facilitate pollination of self-pollinating species is not a new idea and other authors may wish to explore this further as this was beyond the scope of this thesis. Outside of the Orchidaceae, a study (mentioned in the introduction of this

chapter) which took place in Iowa looked at two herbaceous species (*Ranunculus sceleratus* and *Potentilla rivali*) and found a greatly reduced seed set when thrips and aphids were excluded from flowers (Baker & Cruden 1991). This reduction occurred regardless of whether other floral visitors were also excluded from visiting experimental flowers (although it was much greater when both were excluded), and appeared to suggest that aphids and thrips were responsible for up to half of all pollination when larger visitors were not present. Observations confirmed that these insects moved pollen between flowers which suggests that species that are frequently believed to be autonomously selfing through exclusion experiments may actually be selfing through the assistance of these species, and thus falsely classified (Baker & Cruden 1991). Because aphids were not excluded from the current experiment, and because aphids were observed on the pollinia of *Gastrodia* 'long column', it is possible that the reduction in fruit set following bagging may have been cushioned by an unacknowledged service provided by these species. However, this is less likely because of the structure of *Gastrodia* 'long column's' pollen which forms clumps that are much more difficult to move than the loose pollen of *Ranunculus sceleratus* and *Potentilla rivali.* Empirical investigations are needed here.

Implications

Many of New Zealand's rare endemic terrestrial orchids are considered threatened (Lehnebach *et al.* 2005) and some conservation implications can be taken from this chapter. Numerous bees were observed visiting *Gastrodia* 'long column' and bagging led to failure to set fruit, suggesting that this species is dependent upon insect vectors for pollination. Natural fruit set is high which indicates that this species is not currently pollen limited; however, species which depend on mutualisms are thought to be at greater risk of extinction through changing environments as a result of the loss of their pollinators (Bond 1994). Therefore any future conservation efforts which focus on *Gastrodia* 'long column' will need to also consider the state of the pollinating insect fauna at target locations. That *Gastrodia* 'long column' relies upon pollinators at all is surprising as many of New Zealand's orchid species are thought to be self-pollinated, and the number of these species may be lower than the 60% estimate made by Molloy (1990). The high levels of pollination success seen above are interesting if native bees are the primary pollinators of this species.

The most interesting question these results raise is that of the state of *Gastrodia* 'long column's' pollination in a native forest compared with that in an urban setting. It would be particularly novel if pollination services in an urban environment are greater than those in a less urban area. Similar studies which expand on the ideas outlined in this chapter would be in a position to address the first two requirements identified by Bond (1994); the risk of pollination failure, and how dependent *Gastrodia* 'long column' is upon insects for successful pollination. Beyond this, further research may wish to concentrate on the interactions between *Gastrodia* species and particular insect pollinators, or perform larger experimental tests to determine the level of *Gastrodia* dependence upon pollinators through exclusions of both large *and* small pollinators (i.e. aphids). This will hopefully lead to more accurate distinctions between autonomous and insect-facilitated self-pollination. By continuing to expand current knowledge of *Gastrodia* species a more holistic understanding of New Zealand's flora and pollinating fauna can be achieved. By doing so conservation efforts could be redirected to prevent or mitigate possible losses of this species.

Chapter 5

Synthesis

5.1 Patterns in flower visitation of flying insects in urban Christchurch

The small, relatively simple structures of many of New Zealand's flowers are thought to be the result of a fairly unspecialised pollination system (Lloyd 1985). Flowers are frequently dioecious, radially symmetrical, and dish shaped with pollen and receptive stigmas readily exposed. These attributes are all thought traits of an imprecise pollinating service which asks little of its pollinators (Lloyd 1985). Colours tend towards white, even in species with brightly coloured close relatives elsewhere in the world, and flowers are commonly small (a trait often attributed to selfing or wind pollinated systems) despite an at least equal reliance upon outcrossing as found in pollination systems elsewhere in the world (Lloyd 1985). These traits are thought to have combined as a result of generalist insect pollinators which visit a broad range of plant species (Lloyd 1985). Insect visitors in turn were thought to lack strong preferences, and visit simple, easily accessible flowers.

Recently, this idea of lack of pollinator preference has been challenged. Campbell *et al.* (2010) found that native invertebrate pollinators showed preferences for particular flower species in an alpine environment, and these preferences were sometimes also determined by floral colouration. I have shown that New Zealand's native and exotic insect pollinators' relative visitation can be affected by a plant's provenance and the colour of its flowers when observed in uncontrolled urban systems (Chapter 2). When insects were grouped according to provenance, native pollinators visited native white flowers relatively more often than coloured exotic flowers, while exotic pollinators showed greater relative visitation to coloured exotic plant species. As mentioned in chapter 2, the preference of native or exotic pollinators for native or exotic plants will be for a trait (or group of traits) common to the plant's provenance as there is no such thing as a native or exotic trait. This suggests that pollinator preference may have been a driving force of the white colouration of many native flowers seen in New Zealand's current flora. The often white coloration of the flora may undergo changes with the arrival and establishment of exotic insect pollinators. Exotic pollinators visit exotic coloured flowers comparatively more often than native white flowers, and *Apis mellifera* and *Bombus* species visit purple over white flowers (Chapter 2). If these pollinators reach sufficient numbers they may then drive floral evolution towards a greater inclusion of exotic plant species with bright colourful displays. The patterns in relative insect visitation that I have shown may have been driven, in part, by a preference for floral species as was the case in Campbell *et al.*' (2010) work. This is because an experimental test of these results failed to show any pollinator preferences using a few selected plant species (Chapter 3). Therefore it is likely that New Zealand's flower visiting insects (both native and exotic) have preferences in regards to a number of different factors such as colour, provenance, and species, making generalisations across insect species difficult.

The relative abundance of insect pollinators changes, even on short temporal spatial scale. Instantaneous sampling between January and April 2012 showed a difference in the relative abundance of native and exotic insect pollinators at sites along a gradient of urbanisation, with some species found at only one or two of the three sites. Exotic pollinators are at greater relative abundances to natives in urban communities, with native insect pollinators at greater relative

abundance at sites with secondary native forest (Chapter 2). This is important to note because it demonstrates the effect land use change is having on flower visiting insect species, and may wish to be considered in council mediated mitigating strategies. However, native insects were still able to provide an adequate pollination service to the inconspicuous plant species *Gastrodia* 'long column' (Chapter 4).

Implications for management

The preferences of New Zealand's flower visiting insects can help inform conservation managers as it can be predicted which plants will be preferred by which groups of pollinators. From this, flowers can then be selected which facilitate faunal establishment. In this case native white flowers would likely be the most general option to increase native insect pollinator numbers if no particular insect species is being targeted, and an avoidance of bright colours such as purple would be suggested if exotic species (i.e. *Apis mellifera* or *Bombus* taxa) are not wanted.

Indirect drivers of petal colouration in New Zealand

Other possible drivers of petal colouration should also be considered. It is accepted that pollinators are often the driving force behind selection on floral pigmentation (Armbruster 2002). However, pleiotropic relationships may mean that indirect factors may be influencing the selection for white flowers, such as drought tolerance, protection from herbivory and UV-B, greater pollen production, or increased fertilization success (McKee & Richards 1998; Schemske & Bierzychudek 2001; Armbruster 2002; Coberly & Rausher 2003; Gould 2004). This is particularly the case when petal colour is linked to the pigments found in vegetative tissues (Dick *et al.* 2011). Relationships between flower colour and vegetative effects are thought widespread, and some authors suggest that pleiotropic relationships are more likely drivers of floral evolution in some plant families than direct selection by pollinators (Armbruster 2002). *Linanthus parryae* is a polymorphic angiosperm with white or blue flowers (Schemske & Bierzychudek 2001). These colourations have been shown to correlate with seed production in Los Angeles County, California, with white achieving higher numerical success in years of high seed production, and blue flowers being more successful in lower years (Schemske & Bierzychudek 2001). The authors here were unable to ascertain why these different morphs had varying success. There was no correlation with either pollinator visitation rate or efficiency of water use. However they did conclude that selection, rather than genetic drift, was driving these polymorphisms. While pollinators are a major driver of petal colour selection, other factors are often of equal but lesser known importance.

Another possible indirect driver of petal colouration is anthocyanin pigments. Anthocyanins are flavonoid pigments which can be found in all plant tissues. These pigments lead to the costly red colouration often seen in the leaves of deciduous trees (Gould 2004). Scientists have asked why plants would spend so much energy producing an apparently useless colouration. However, these pigments have recently been shown to help reduce the herbivory and disease a plant suffers, increase resistance to UV-B, and improve drought tolerance (Gould 2004). One such early European study found that polymorphic flowers which contained anthocyanin pigments performed equally well to those without in normal conditions. However under drought stress those with anthocyanins found in any of the plant tissue performed markedly better (Warren & MacKenzie 2001). Therefore

indirect stresses may drive colouration, rather than more obvious direct drivers such as pollinator visitation.

5.2 The importance of pollinators

Pollinator declines worldwide

Pollinators are declining worldwide (Burkle *et al.* 2013; Tylianakis 2013). This is both an agricultural and conservation issue as pollinators are important in the success of food crops (Howlett & Donovan 2010) and species-rich areas near crops are important in supplementing pollination by increasing wild-pollinator numbers (Isaacs & Tuell 2007)**.** A study which examined disruptions to plant-pollinator interactions in a single Illinois system over 120 years found that half of the bee species had undergone some level of change (Burkle *et al.* 2013). These changes led to degradation of pollination quality and quantity, as well as mismatched pollination systems and possible plant extinctions. Changes to pollinating species have historically been absorbed by the resilience of community systems. However the authors suggest that this resilience is decreasing and may not continue in the future (Burkle *et al.* 2013). Because pollination is a highly complex system, indirect effects from global change are often unknown (Laliberte & Tylianakis 2012).

The importance of pollinators in New Zealand

Bond's (1994) criteria for assessing the risk of plant extinctions has three aspects; probability of pollination failure, dependence upon mutualisms, and a plant's dependence upon seeds (Chapter 4). Plants which depend upon pollinators for successful reproduction are more at a risk of pollination failure (Bond 1994). Therefore it is important to determine the reproductive systems of a wide range of New Zealand's plant species so that an assessment of the risk of failure can be made. *Gastrodia* 'long column' (Chapter 4) may depend upon insect pollinators for successful fruit set. This plant species was visited by native insect pollinators; particularly *Lasioglossum* species. *Lasioglossum* bees appear to be at sufficiently high abundances for pollination services to *Gastrodia* 'long column' in Christchurch based on the successful fruit set of open flowers of *Gastrodia* 'long column' (at least 75%, Chapter 4), and observation counts at a nearby location (Chapters 2 and 3). Therefore at this time it is unlikely that *Gastrodia* 'long column' is at risk of pollination failure, but its high dependence upon pollinators for successful fruit set mean that it is a species that should be considered when risk assessments of mutualistic breakdowns of New Zealand's plant-pollinators are done in the future. This also demonstrates that inconspicuous plants which may not appear to be pollinator-dependent (as a closely related species, *Gastrodia cunninghamii,* has been shown to autonomously self in a North Island study (Lehnebach *et al.* 2005)) may rely on pollinators for successful reproduction, emphasising the importance of pollinators in New Zealand.

Insect pollinators are important in New Zealand as a number of species depend upon them for successful reproduction. This is a result of a number of factors such as spatial or temporal mechanisms which prevent self-fertilization or sexual dimorphism (explored further in Chapter 1) (Newstrom & Robertson 2005). Within this, some insect species are more important to plants than others as a result of relative visitation rate and effectiveness in moving and depositing pollen (Bischoff *et al.* 2012). In a study conducted in the Remarkables Range in Otago, New Zealand; *Ourisia glandulosa* was visited equally by *Hylaeus matamoko* and *Allograpta* species (endemic bee and

syrphid flies) while *Wahlenbergia albomarginata* was predominantly visited by *Hylaeus matamoko* (Bischoff *et al.* 2012). Further, the much greater effectiveness of *Hylaeus matamoko* led to estimations of performing approximately 90% of the pollination of *Ourisia glandulosa* and 95% of the pollination of *Wahlenbergia albomarginata.* This demonstrates the importance a single insect species can have in the pollination of some plant species in New Zealand.

5.3 Are pollinators able to replace *Apis mellifera?*

Apis mellifera is declining in New Zealand as a result of the establishment of the varroa mite (*Varroa destructor)* (Howlett & Donovan 2010), and declining overseas as a result of Colony Collapse Disorder (Rader *et al.* 2009). It is imperative that alternative provider(s) of pollination services are found to supplement existing services before economic impacts are felt. Currently, both wild and managed pollinators contribute to crop pollination. Management of native bees on farmland to improve agricultural pollination requires an understanding of the bee species targeted (Isaacs & Tuell 2007). Sufficient provisions of required resources such as nesting sites and materials, food when crops are not in bloom, access to water, and the disuse (or careful selection) of pesticides, is also required (Greer 1999; Isaacs & Tuell 2007).

However, *Apis mellifera* may not be the superior pollinator as is commonly thought. Interestingly, a worldwide study from 600 fields across all continents except Antarctica served to negate the notion that *Apis mellifera* is the best pollinating species (Garibaldi *et al.* 2013). The authors found that only 14% of the systems surveyed had a significant increase in fruit set following honey bee visitation (Garibaldi *et al.* 2013). This idea of the overstated importance of *Apis mellifera* has been proposed elsewhere (Ollerton *et al.* 2012), but rather than diminishing the importance of *Apis mellifera*, the importance of other pollinators has perhaps been understated. Wild pollinators were also found to be twice as effective in their pollination as *Apis mellifera* when considered for the same number of visits (Garibaldi *et al.* 2013). Because managed honey bees and wild pollinators behaved independently they served to complement rather than replace one another, and so informed management in the future may wish to concentrate on enhancing both managed and unmanaged pollinator numbers (Garibaldi *et al.* 2013).

Therefore, it is essential to protect wild pollinators because wild pollinators have been shown to consistently supplement crop pollination in other systems (Garibaldi *et al.* 2013). The use of solitary bees as alternative pollinators has been investigated offshore. This pollination alternative has a number of advantages. Pollinators are active and abundant earlier in the season, rapid brief flight leads to more plants visited in a single area, and bees tend to have a gentle (or no) sting so less of a nuisance to farmers (Greer 1999). Alternative pollinator visitation does tend to be less generalised than that *Apis mellifera*, and so farmers will need to understand which pollinator would suit their needs and implement strategies to increase local abundance (Greer 1999). One study which investigated whether managed and unmanaged pollinators were equally effective pollinators of 'Pak Choi', *Brassica rapa* var. *chinensis*, in New Zealand found that while the two exotic species, *Eristalis tenax and Bombus terrestris,* and the native *Leioproctus* were similarly effective in terms of visitation rate and pollen disposition; *Apis mellifera* still accomplished the majority of pollination through sheer numbers (Rader *et al.* 2009). This is encouraging as it implies that should the numbers of unmanaged pollinators reach those of *Apis mellifera*, pollination failure in agriculture is unlikely.

This result was furthered with a larger New Zealand study conducted over four years which again assessed variation in pollinator effectiveness. The authors found that in two of the four years, unmanaged pollinators were similarly (if not more) effective in terms of pollination services compared to *Apis mellifera* (Rader *et al.* 2012), and this consistency over longer periods is encouraging. With greater understanding of pollinator preferences a possible alternative to *Apis mellifera* may be found with minimal damage to national and local economies. The findings of this thesis suggest that native pollinators may be a viable option to pollinate crops with white flowers although further investigation is needed.

5.4 The role of exotic mutualists in New Zealand ecosystems

A number of exotic mutualists are established in New Zealand and are therefore likely to be playing a pollinating role in New Zealand's ecosystems. Exotic insect pollinators are at relatively high abundances in Christchurch city (Chapter 3), and therefore their role in pollination in the city is likely to be important. While assessing the effectiveness of different pollinators was beyond the scope of this study, exotic pollinating species outnumbered native invertebrates at two of the three sites observed (Chapter 3). Therefore, combined with their large, furry bodies, it is reasonable to assume that they may be performing the bulk of pollination services in urban areas. Because *Apis mellifera* and *Bombus* species prefer purple over white flowers (Chapter 3) they may not play a great role in the pollination of the New Zealand native flora which has small, white flowers. However, while grouped exotic species showed a preference for exotic coloured flowers, *Apis mellifera* and *Bombus* taxa did not show a significant preference for either native or exotic flowers. Their preferences for coloured flowers may result in changes to the composition of the native flora over time. However, as they are predominantly found in urban areas these changes may be restricted to zones of high disturbance.

5.5 The relationship between New Zealand plants and their pollinators

Pigmentation diversity, both within and between species, is commonplace in angiosperm evolution (Dick *et al.* 2011), although in New Zealand flowers are often small, simple and white (Wardle 1978; Lloyd 1985; Newstrom & Robertson 2005; Campbell *et al.* 2010, 2012). Whether this is a result of pollinator visitation or indirect effects is uncertain, with some authors going so far as to say that New Zealand's pollinators have no preferences in regards to colour (Wardle 1978). Chapter 3 attempted to test this concept to determine whether New Zealand's pollinators have colour preferences, whether changes to floral composition are likely with the introduction of exotic invertebrate pollinators, and whether pollinator preferences have been the main driver of New Zealand's often white floral colourations. New Zealand's pollinators do have preferences, however these are contextual and therefore difficult to generalise. Because preferences exist, the introduction of exotic invertebrate flower visiting species is likely to have some change to the composition of the existing flora as current preferences have likely played a role in selecting for the plants seen today. However because introduced pollinating insects are not as abundant in native-dominated areas changes to the traits selected for may be restricted to disturbed and urban locations. It helps from a conservation perspective to learn these preferences as management strategies can plant targeted species which are preferred by native pollinators. Because pollinator preferences are complex it is not yet possible to make claims in regards to which would be the main

driver of floral evolution in New Zealand, however it is likely that they have influenced colouration to some extent, along with other factors such as pleiotropic effects, and therefore the next step would be quantifying this role.

5.6 Future research

It would be useful to follow up my work on *Gastrodia* 'long column' in other seasons and places because I was only able to work on 11 flower stalks at one location in the 2012 season, as explained in the methods section of Chapter 4. This would determine whether pollinators other than *Lasioglossum* bees are frequent visitors to this orchid.

Future research into the colouration of New Zealand's flora may wish to concentrate on the possible direct and indirect selective forces. Insect pollinator preferences are not the only drivers of selection on plants, as pleiotropic traits such as carotenoids (yellow-orange colouration) or protection from herbivores can also have a great influence (Menzel 1979; Chittka & Thomson 2001). Pollinator preferences may be influenced by a number of single or correlated traits such as colour, size, shape, scent, species, and symmetry (Ackerman *et al.* 1997; Chittka & Thomson 2001; Campbell *et al.* 2010; Dyer *et al.* 2012; Hirota *et al.* 2012; Kaczorowski *et al.* 2012); and are perhaps a result of learned rewards or improved foraging efficiency (Waser & Price 1983; Chittka *et al.* 1999; Chittka & Thomson 2001). These possibly correlated traits need to be decoupled so that the underlying mechanisms of pollinator choice can be determined. Including exotic species is an important step because these species have a number of wild colonies and so therefore will also be driving future selection on the New Zealand flora. It would be interesting to compare the preferences of exotic insect pollinators in New Zealand to those in their homeland to determine whether introduced species are adapting their preferences to fit with New Zealand's unique flora (and therefore may be supplementing insect pollination in New Zealand) or whether they are retaining strong preferences and changing the direction of New Zealand's floral evolution. This paves the way towards a new understanding of New Zealand's native and exotic flower visiting insects and raises new questions into the origins of the simplicity of New Zealand's native flora. This data can be combined so inferences around conservation, agriculture, ecology, and evolution can be made such as the likelihood of weed establishment, and possible pollination supplementation by fauna other than *A. mellifera*. My findings on pollinator preferences need to be more firmly established under different conditions for generalizations to be confidently made.

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